(19) World Intellectual Property Organization International Bureau



(43) International Publication Date 21 March 2002 (21.03.2002)

PCT

(10) International Publication Number WO 02/22080 A2

(51) International Patent Classification7:

A61K

- (21) International Application Number: PCT/US01/28861
- (22) International Filing Date:

14 September 2001 (14.09.2001)

(25) Filing Language:

English

(26) Publication Language:

English

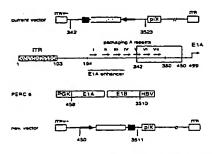
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- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, 7W
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian

[Continued on next page]

(54) Title: ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS



Modifications made to the current adenovactor backbone in the generation of the ner

(57) Abstract: First generation adenoviral vectors and associated recombinant adenovirus-based HIV vaccines which show enhanced stability and growth properties and greater cellular-mediated immunity are described within this specification. These adenoviral vectors are utilized to generate and produce through cell culture various adenoviral-based HIV-1 vaccines which contain HIV-1 gag, HIV-1 pol and/or HIV-1 nef polynucleotide pharmaceutical products, and biologically relevant modifications thereof. These adenovirus vaccines, when directly introduced into living vertebrate tissue, preferably a mammalian host such as a human or a non-human mammal of commercial or domestic veterinary importance, express the HIV1-Gag, Pol and/or Nef protein or biologically modification thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding HIV-1 Gag, encoding codon optimized HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNAse H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The adenoviral vaccines of the present invention, when administered alone or in a combined modality regime, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.



patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Published:

 without international search report and to be republished upon receipt of that report

TITLE OF THE INVENTION

ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS

5 CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit, under 35 U.S.C. §119(e), of U.S. provisional applications 60/233,180, 60/279,056, and Attorney Docket 20867PV2 (serial number unassigned), filed September 15, 2000, March 27, 2001, and September 7, 2001, respectively.

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STATEMENT REGARDING FEDERALLY-SPONSORED R&D Not Applicable

REFERENCE TO MICROFICHE APPENDIX

15 Not Applicable

FIELD OF THE INVENTION

The present invention relates to recombinant, replication-deficient first generation adenovirus vaccines found to exhibit enhanced growth properties and greater cellular-mediated immunity as compared to other replication-deficient vectors. The invention also relates to the associated first generation adenoviral vectors described herein, which, through the incorporation of additional 5' adenovirus sequence, enhance large scale production efficiency of the recombinant, replicationdefective adenovirus described herein. Another aspect of the instant invention is the surprising discovery that the intron A portion of the human cytomegalovirus (hCMV) promoter constitutes a region of instability in adenoviral vector constructs. Removal of this region from adenoviral expression constructs results in greatly improved vector stability. Therefore, improved vectors expressing a transgene under the control of an intron A-deleted CMV promoter constitute a further aspect of this invention. These adenoviral vectors are useful for generating recombinant adenovirus vaccines against human immunodeficiency virus (HTV). In particular, the first generation adenovirus vectors disclosed herein are utilized to construct and generate adenovirus-based HIV-1 vaccines which contain HIV-1 Gag, HIV-1 Pol and/or HIV-1 Nef polynucleotide pharmaceutical products, and biologically active modifications thereof. Host administration of the recombinant, replication-deficient adenovirus vaccines described herein results in expression of HIV-1 Gag, HIV-1- Pol and/or Nef protein or

immunologically relevant modifications thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding codon optimized HIV-1 Gag, HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNAse H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef, and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The HIV adenovirus vaccines of the present invention, when administered alone or in a combined modality and/or prime/boost regimen, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.

BACKGROUND OF THE INVENTION

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Human Immunodeficiency Virus-1 (HIV-1) is the etiological agent of acquired human immune deficiency syndrome (AIDS) and related disorders. HIV-1 is an RNA virus of the Retroviridae family and exhibits the 5'LTR-gag-pol-env-LTR 3'organization of all retroviruses. The integrated form of HIV-1, known as the provirus, is approximately 9.8 Kb in length. Each end of the viral genome contains flanking sequences known as long terminal repeats (LTRs). The HIV genes encode at least nine proteins and are divided into three classes; the major structural proteins (Gag, Pol, and Env), the regulatory proteins (Tat and Rev); and the accessory proteins (Vpu, Vpr, Vif and Nef).

The gag gene encodes a 55-kilodalton (kDa) precursor protein (p55) which is expressed from the unspliced viral mRNA and is proteolytically processed by the HIV protease, a product of the pol gene. The mature p55 protein products are p17 (matrix), p24 (capsid), p9 (nucleocapsid) and p6.

The pol gene encodes proteins necessary for virus replication; a reverse transcriptase, a protease, integrase and RNAse H. These viral proteins are expressed as a Gag-Pol fusion protein, a 160 kDa precursor protein which is generated via a ribosomal frame shifting. The viral encoded protease proteolytically cleaves the Pol polypeptide away from the Gag-Pol fusion and further cleaves the Pol polypeptide to the mature proteins which provide protease (Pro, P10), reverse transcriptase (RT, P50), integrase (IN, p31) and RNAse H (RNAse, p15) activities.

The *nef* gene encodes an early accessory HIV protein (Nef) which has been shown to possess several activities such as down regulating CD4 expression, disturbing T-cell activation and stimulating HIV infectivity.

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The *env* gene encodes the viral envelope glycoprotein that is translated as a 160-kilodalton (kDa) precursor (gp160) and then cleaved by a cellular protease to yield the external 120-kDa envelope glycoprotein (gp120) and the transmembrane 41-kDa envelope glycoprotein (gp41). Gp120 and gp41 remain associated and are displayed on the viral particles and the surface of HIV-infected cells.

The *tat* gene encodes a long form and a short form of the Tat protein, a RNA binding protein which is a transcriptional transactivator essential for HIV-1 replication.

The rev gene encodes the 13 kDa Rev protein, a RNA binding protein. The Rev protein binds to a region of the viral RNA termed the Rev response element (RRE). The Rev protein promotes transfer of unspliced viral RNA from the nucleus to the cytoplasm. The Rev protein is required for HIV late gene expression and in turn, HIV replication.

Gp120 binds to the CD4/chemokine receptor present on the surface of helper T-lymphocytes, macrophages and other target cells in addition to other co-receptor molecules. X4 (macrophage tropic) virus show tropism for CD4/CXCR4 complexes while a R5 (T-cell line tropic) virus interacts with a CD4/CCR5 receptor complex. After gp120 binds to CD4, gp41 mediates the fusion event responsible for virus entry. The virus fuses with and enters the target cell, followed by reverse transcription of its single stranded RNA genome into the double-stranded DNA via a RNA dependent DNA polymerase. The viral DNA, known as provirus, enters the cell nucleus, where the viral DNA directs the production of new viral RNA within the nucleus, expression of early and late HIV viral proteins, and subsequently the production and cellular release of new virus particles. Recent advances in the ability to detect viral load within the host shows that the primary infection results in an extremely high generation and tissue distribution of the virus, followed by a steady state level of virus (albeit through a continual viral production and turnover during this phase), leading ultimately to another burst of virus load which leads to the onset of clinical AIDS. Productively infected cells have a half life of several days, whereas chronically or latently infected cells have a 3-week half life, followed by non-productively infected cells which have a long half life (over 100 days) but do not significantly contribute to day to day viral loads seen throughout the course of disease.

Destruction of CD4 helper T lymphocytes, which are critical to immune defense, is a major cause of the progressive immune dysfunction that is the hallmark of HIV infection. The loss of CD4 T-cells seriously impairs the body's ability to fight most invaders, but it has a particularly severe impact on the defenses against viruses, fungi, parasites and certain bacteria, including mycobacteria.

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Effective treatment regimens for HIV-1 infected individuals have become available recently. However, these drugs will not have a significant impact on the disease in many parts of the world and they will have a minimal impact in halting the spread of infection within the human population. As is true of many other infectious diseases, a significant epidemiologic impact on the spread of HIV-1 infection will only occur subsequent to the development and introduction of an effective vaccine. There are a number of factors that have contributed to the lack of successful vaccine development to date. As noted above, it is now apparent that in a chronically infected person there exists constant virus production in spite of the presence of anti-HIV-1 humoral and cellular immune responses and destruction of virally infected cells. As in the case of other infectious diseases, the outcome of disease is the result of a balance between the kinetics and the magnitude of the immune response and the pathogen replicative rate and accessibility to the immune response. Pre-existing immunity may be more successful with an acute infection than an evolving immune response can be with an established infection. A second factor is the considerable genetic variability of the virus. Although anti-HIV-1 antibodies exist that can neutralize HIV-1 infectivity in cell culture, these antibodies are generally virus isolate-specific in their activity. It has proven impossible to define serological groupings of HIV-1 using traditional methods. Rather, the virus seems to define a serological "continuum" so that individual neutralizing antibody responses, at best, are effective against only a handful of viral variants. Given this latter observation, it would be useful to identify immunogens and related delivery technologies that are likely to elicit anti-HIV-1 cellular immune responses. It is known that in order to generate CTL responses antigen must be synthesized within or introduced into cells, subsequently processed into small peptides by the proteasome complex, and translocated into the endoplasmic reticulum/Golgi complex secretory pathway for eventual association with major histocompatibility complex (MHC) class I proteins. CD8⁺ T lymphocytes recognize antigen in association with class I MHC via the T cell receptor (TCR) and the CD8 cell surface protein. Activation of naive CD8⁺ T cells into activated effector or memory cells generally requires both TCR engagement of antigen as described above as well as engagement of costimulatory proteins. Optimal

induction of CTL responses usually requires "help" in the form of cytokines from CD4⁺ T lymphocytes which recognize antigen associated with MHC class II molecules via TCR and CD4 engagement.

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European Patent Applications 0 638 316 (Published February 15, 1995) and 0 586 076 (Published March 9, 1994), (both assigned to American Home Products Corporation) describe replicating adenovirus vectors carrying an HIV gene, including env or gag. Various treatment regimens were used with chimpanzees and dogs, some of which included booster adenovirus or protein plus alum treatments.

Replication-defective adenoviral vectors harboring deletions in the E1 region are known, and recent adenoviral vectors have incorporated the known packaging repeats into these vectors; e.g., see EP 0 707 071, disclosing, *inter alia*, an adenoviral vector deleted of E1 sequences from base pairs 459 to 3328; and U.S. Patent No. 6,033,908, disclosing, *inter alia*, an adenoviral vector deleted of base pairs 459-3510. The packaging efficiency of adenovirus has been taught to depend on the number of incorporated individual A (packaging) repeats; *see*, *e.g.*, Gräble and Hearing, 1990 *J. Virol*. 64(5):2047-2056; Gräble and Hearing, 1992 *J. Virol*. 66(2):723-731.

Larder, et al., (1987, *Nature* 327: 716-717) and Larder, et al., (1989, *Proc. Natl. Acad. Sci.* 86: 4803-4807) disclose site specific mutagenesis of HIV-1 RT and the effect such changes have on *in vitro* activity and infectivity related to interaction with known inhibitors of RT.

Davies, et al. (1991, *Science* 252:, 88-95) disclose the crystal structure of the RNase H domain of HIV-1 Pol.

Schatz, et al. (1989, FEBS Lett. 257: 311-314) disclose that mutations Glu478Gln and His539Phe in a complete HIV-1 RT/RNase H DNA fragment results in defective RNase activity without effecting RT activity.

Mizrahi, et al. (1990, *Nucl. Acids. Res.* 18: pp. 5359-5353) disclose additional mutations Asp443Asn and Asp498Asn in the RNase region of the *pol* gene which also results in defective RNase activity. The authors note that the Asp498Asn mutant was difficult to characterize due to instability of this mutant protein.

Leavitt, et al. (1993, J. Biol. Chem. 268: 2113-2119) disclose several mutations, including a Asp64Val mutation, which show differing effect on HIV-1 integrase (IN) activity.

Wiskerchen, et al. (1995, *J. Virol.* 69: 376-386) disclose singe and double mutants, including mutation of aspartic acid residues which effect HIV-1 IN and viral replication functions.

It would be of great import in the battle against AIDS to produce a prophylactic- and/or therapeutic-based HIV vaccine which generates a strong cellular immune response against an HIV infection. The present invention addresses and meets these needs by disclosing a class of adenovirus vaccines which, upon host administration, express codon optimized and modified versions of the HIV-1 genes, gag, pol and nef. These recombinant, replication-defective adenovirus vaccines may be administered to a host, such as a human, alone or as part of a combined modality regimen and/or prime-boost vaccination regimen with components of the present invention and/or a distinct viral HIV DNA vaccine, non-viral HIV DNA vaccine, HIV subunit vaccine, an HIV whole killed vaccine and/or a live attenuated HIV vaccine.

SUMMARY OF THE INVENTION

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The present invention relates to enhanced replication-defective recombinant adenovirus vaccine vectors and associated recombinant, replication-deficient adenovirus vaccines which encode various forms of HIV-1 Gag, HIV-1 Pol, and/or HIV-1 Nef, including immunologically relevant modifications of HIV-1 Gag, HIV-1 Pol and HIV-1 Nef. The adenovirus vaccines of the present invention express HIV antigens and provide for improved cellular-mediated immune responses upon host administration. Potential vaccinees include but are not limited to primates and especially humans and non-human primates, and also include any non-human mammal of commercial or domestic veterinary importance. An effect of the improved recombinant adenovirus-based vaccines of the present invention should be a lower transmission rate to previously uninfected individuals (i.e., prophylactic applications) and/or reduction in the levels of the viral loads within an infected individual (i.e., therapeutic applications), so as to prolong the asymptomatic phase of HIV-1 infection. In particular, the present invention relates to adenoviral-based vaccines which encode various forms of codon optimized HIV-1 Gag (including but in no way limited to p55 versions of codon optimized full length (FL) Gag and tPA-Gag fusion proteins), HIV-1 Pol, HIV-1 Nef, and selected modifications of immunological relevance. The administration, intracellular delivery and expression of these adenovirus vaccines elicit a host CTL and Th response. The preferred replication-defective recombinant adenoviral vaccine vectors include but are not limited to synthetic DNA molecules which (1) encode codon optimized versions of wild type HIV-1 Gag; (2) encode codon optimized versions of HIV-1 Pol; (3) encode codon optimized versions of HIV-1 Pol fusion proteins; (4) encode codon optimized versions of modified HIV-1 Pol proteins and fusion proteins, including but not limited

to pol modifications involving residues within the catalytic regions responsible for RT, RNase and IN activity within the host cell; (5) encode codon optimized versions of wild type HIV-1 Nef; (6) codon optimized versions of HIV-1 Nef fusion proteins; and/or (7) codon optimized versions of HIV-1 Nef derivatives, including but not limited to nef modifications involving introduction of an amino-terminal leader sequence, removal of an amino-terminal myristylation site and/or introduction of dileucine motif mutations. The Nef-based fusion and modified proteins, disclosed within this specification and expressed from an adenoviral-based vector vaccine this specification, may possess altered trafficking and/or host cell function while retaining the ability to be properly presented to the host MHC I complex and in turn elicit a host CTL and Th response. Examples of HIV-1 Gag, Pol and/or Nef fusion proteins include but are not limited to fusion of a leader or signal peptide at the NH₂-teriminal portion of the viral antigen coding region. Such a leader peptide includes but is not limited to a tPA leader peptide.

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The adenoviral vector utilized in construction of the HIV-1 Gag-, HIV-1 Poland/or HIV-1 Nef- based vaccines of the present invention may comprise any replication-defective adenoviral vector which provides for enhanced genetic stability of the recombinant adenoviral genome through large scale production and purification of the recombinant virus. In other words, an HIV-1 Gag-, Pol- or Nef-based adenovirus vaccine of the present invention is a purified recombinant, replicationdefective adenovirus which is shown to be genetically stable through multiple passages in cell culture and remains so during large scale production and purification procedures. Such a recombinant adenovirus vector and harvested adenovirus vaccine lends itself to large scale dose filling and subsequent worldwide distribution procedures which will be demanded of an efficacious monovalent or multivalent HIV vaccine. The present invention meets this basic requirement with description of a replication-defective adenoviral vector and vectors derived therefrom, at least partially deleted in E1, comprising a wildtype adenovirus cis-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 of the wildtype adenovirus genome. A preferred embodiment of the instant invention comprises base pairs 1-450 of a wildtype adenovirus. In other preferred embodiments, the replication -defective adenoviral vector has, in addition thereto, a region 3' to the E1-deleted region comprising base pairs 3511-3523. Basepairs 342-450 (more particularly, 400-450) constitute an extension of the 5'region of previously disclosed vectors carrying viral antigens, particularly HIV antigens (see, e.g., PCT International Application PCT/US00/18332, published

January 11, 2001 (WO 01/02067), which claims priority to U.S. Provisional Application Serial Nos. 60/142,631 and 60/148,981, filed 7/6/1999 and 8/13/1999, respectively; these documents herein incorporated by reference. Applicants have found that extending the 5' region further into the E1 gene into the disclosed vaccine vectors incorporated elements found to be important in optimizing the packaging of the virus.

As compared to previous vectors not comprising basepairs from about 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 of the wildtype adenovirus genome, vectors comprising the above region exhibited enhanced growth characteristics, with approximately 5-10 fold greater amplification rates, a more potent virus effect, allowing lower doses of virus to be used to generate equivalent immunity; and a greater cellular-mediated immune response than replication-deficient vectors not comprising this region (basepairs 1-450). Even more important, adenoviral constructs derived therefrom are very stable genetically in large-scale production, particularly those comprising an expression cassette under the control of a hCMV promoter devoid of intron A. This is because Applicants have surprisingly found that the intron A portion of the hCMV promoter constituted a region of instability when employed in adenoviral vectors. Applicants have, therefore, identified an enhanced adenoviral vector which is particularly suited for use in gene therapy and nucleotide-based vaccine-vectors which, favorably, lends itself to large scale propagation.

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A preferred embodiment of this invention is a replication-defective adenoviral vector in accordance with the above description wherein the gene is inserted in the form of a gene expression cassette comprising (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and, (c) a transcription terminator.

In preferred embodiments, the E1 gene, other than that contained within basepairs 1-450 or, alternatively, that contained within base pairs 1-450 and 3511-3523 has been deleted from the adenoviral vector, and the gene expression cassette has replaced the deleted E1 gene. In other preferred embodiments, the replication defective adenovirus genome does not have a functional E3 gene, or the E3 gene has been deleted. Most preferably, the E3 region is present within the adenoviral genome. Further preferred embodiments are wherein the gene expression cassette is in an E1 anti-parallel (transcribed in a 3' to 5' direction relative to the vector backbone)

orientation or, more preferably, an E1 parallel (transcribed in a 5' to 3' direction relative to the vector backbone) orientation.

Further embodiments relate to a shuttle plasmid vector comprising: an adenoviral portion and a plasmid portion, wherein said adenovirus portion comprises: 5 a) a replication defective adenovirus genome, at least partially deleted in E1, comprising a wildtype adenovirus cis-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 (preferably, 1-450) of the wildtype adenovirus genome and, preferably, in addition thereto, basepairs 3511-3523 of a wildtype adenovirus sequence; and b) a gene expression cassette comprising: (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and (c) a transcription terminator and/or a polyadenylation site.

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Other aspects of this invention include a host cell comprising said adenoviral vectors and/or said shuttle plasmid vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and (b) harvesting the resultant adenoviral vectors.

To this end, the present invention particularly relates to harvested recombinant, replication defective virus derived from a host cell, such as but not limited to 293 cells or PER.C6® cells, including but not limited to harvested virus related to any of the MRKAd5 vector backbones, with or without an accompanying transgene, including but not limited to the HIV-1 antigens described herein. An HTV-1 vaccine is represented by any harvested, recombinant adenovirus material which expresses any one or more of the HIV-1 antigens disclosed herein. This harvested material may then be purified, formulated and stored prior to host administration.

Another aspect of this invention is a method of generating a cellular immune response against a protein in an individual comprising administering to the individual an adenovirus vaccine vector comprising:

a) a recombinant, replication defective adenoviral vector, at least partially deleted in E1, comprising a wildtype adenovirus cis-acting adenovirus packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 (preferably, 1-450) and, preferably in addition thereto, base pairs 3511-3523 of a wildtype adenovirus sequence, and,

b) a gene expression cassette comprising:(i) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (ii) a heterologous promoter operatively linked to the nucleic acid of part a); and (iii) a transcription terminator and/or a polyadenylation site.

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In view of the efficacious nature of the adenoviral and/or DNA plasmid vaccines described herein, the present invention relates to all methodology regarding administration of one or more of these adenoviral and/or DNA plasmid vaccines to provide effective immunoprophylaxis, to prevent establishment of an HIV-1 infection following exposure to this virus, or as a post-HIV infection therapeutic vaccine to mitigate the acute HIV-1 infection so as to result in the establishment of a lower virus load with beneficial long term consequences. As discussed herein, such a treatment regimen may include a monovalent or multivalent composition, various combined modality applications, and/or a prime/boost regimen to as to optimize antigen expression and a concomitant cellular-mediated and/or humoral immune response upon inoculation into a living vertebrate tissue. Therefore, the present invention provides for methods of using the adenoviral and/or DNA plasmid vaccines disclosed herein within the various parameters disclosed herein as well as any additional parameters known in the art, which, upon introduction into mammalian tissue induces intracellular expression of the gag, pol and/or nef-based vaccines.

To this end, the present invention relates in part to methods of generating a cellular immune response in a vaccinee, preferably a human vaccinee, wherein the individual is given more than one administration of adenovirus vaccine vector, and it may be given in a regimen accompanied by the administration of a plasmid vaccine. The plasmid vaccine (also referred to herein as a "DNA plasmid vaccine" or "vaccine plasmid" comprises a nucleic acid encoding a protein or an immunologically relevant portion thereof, a heterologous promoter operably linked to the nucleic acid sequence, and a transcription terminator or a polyadenylation signal (such as bGH or SPA, respectively). There may be a predetermined minimum amount of time separating the administrations. The individual can be given a first dose of plasmid vaccine, and then a second dose of plasmid vaccine. Alternatively, the individual may be given a first dose of adenovirus vaccine, and then a second dose of adenovirus vaccine. In other embodiments, the plasmid vaccine is administered first, followed after a time by administration of the adenovirus vaccine. Conversely, the adenovirus vaccine may be administered first, followed by administration of plasmid vaccine after a time. In these embodiments, an individual may be given multiple doses of the same adenovirus serotype in either viral vector or plasmid form, or the virus may be of

differing serotypes. In the alternative, a viral antigen of interest can be first delivered via a viral vaccine other than an adenovirus-based vaccine, and then followed with the adenoviral vaccine disclosed. Alternative viral vaccines include but are not limited to pox virus and venezuelan equine encephilitis virus.

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The present invention also relates to multivalent adenovirus vaccine compositions which comprise Gag, Pol and Nef components described herein; see, e.g., Example 29 and Table 25. Such compositions will provide for an enhanced cellular immune response subsequent to host administration, particularly given the genetic diversity of human MHCs and of circulating virus. Examples, but not limitations, include MRKAd5-vector based multivalent vaccine compositions which provide for a divalent (i.e., gag and nef, gag and pol, or pol and nef components) or a trivalent vaccine (i.e., gag, pol and nef components) composition. Such a mutlivalent vaccine may be filled for a single dose or may consist of multiple inoculations of each individually filled component; and may in addition be part of a prime/boost regimen with viral or non-viral vector vaccines as introduced in the previous paragraph. To this end, preferred compositions are MRKAd5 adenovirus used in combination with multiple, distinct HIV antigen classes. Each HIV antigen class is subject to sequence manipulation, thus providing for a multitude of potential vaccine combinations; and such combinations are within the scope of the present invention. The utilization of such combined modalities vaccine formulation and administration increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

The concept of a "combined modality" as disclosed herein also covers the alternative mode of administration whereby multiple HIV-1 viral antigens may be ligated into a proper shuttle plasmid for generation of a pre-adenoviral plasmid comprising multiple open reading frames. For example, a trivalent vector may comprise a gag-pol-nef fusion, in either a E3(-) or E3(+) background, preferably a E3 deleted backbone, or possibly a "2+1" divalent vaccine, such as a gag-pol fusion (i.e., codon optimized p55 gag and inactivated optimized pol; Example 29 and Table 25) within the same MRKAd5 backbone, with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES). Therefore, a multivalent vaccine delivered as a single, or possibly a second harvested recombinant, replication-deficient adenovirus is contemplated as part of the present invention.

Therefore, the adenoviral vaccines and plasmid DNA vaccines of this invention may be administered alone, or may be part of a prime and boost administration regimen. A mixed modality priming and booster inoculation scheme will result in an enhanced immune response, particularly if pre-existing anti-vector immune responses are present. This one aspect of this invention is a method of priming a subject with the plasmid vaccine by administering the plasmid vaccine at least one time, allowing a predetermined length of time to pass, and then boosting by administering the adenoviral vaccine. Multiple primings typically, 1-4, are usually employed, although more may be used. The length of time between priming and boost may typically vary from about four months to a year, but other time frames may be used. In experiments with rhesus monkeys, the animals were primed four times with plasmid vaccines, then were boosted 4 months later with the adenoviral vaccine. Their cellular immune response was notably higher than that of animals which had only received adenoviral vaccine. The use of a priming regimen may be particularly preferred in situations where a person has a pre-existing anti-adenovirus immune response.

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It is an object of the present invention to provide for enhanced replication-defective recombinant adenoviral vaccine vector backbones. These recombinant adenoviral backbones may accept one or more transgenes, which may be passaged through cell culture for growth, amplification and harvest.

It is a further object to provide for enhanced replication-defective recombinant adenoviral vaccine vectors which encode various transgenes.

It is also an object of the present invention to provide for a harvested recombinant, replication-deficient adenovirus which shows enhanced growth and amplification rates while in combination with increased virus stability after continuous passage in cell culture. Such a recombinant adenovirus is particularly suited for use in gene therapy and nucleotide-based vaccine vectors which, favorably, lends itself to large scale propagation.

To this end, it is an object of the present invention to provide for (1) enhanced replication-defective recombinant adenoviral vaccine vectors as described herein which encode various forms of HIV-1 Gag, HIV-1 Pol, and/or HIV-1 Nef, including immunologically relevant modifications of HIV-1 Gag, HIV-1 Pol and HIV-1 Nef, and (2) harvested, purified recombinant replication-deficient adenovirus generated by passage of the adenoviral vectors of (1) through one or multiple passages through cell culture, including but not limited to passage through 293 cells or PER.C6® cells.

It is also an object of the present invention to provide for recombinant adenovirus harvested by one or multiple passages through cell culture. As relating to recombinant adenoviral vaccine vector, this recombinant virus is harvested and formulated for subsequent host administration.

It is also an object of the present invention to provide for replication-defective adenoviral vectors wherein at least one gene is inserted in the form of a gene expression cassette comprising (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and, (c) a transcription terminator.

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It is also an object of the present invention to provide for a host cell comprising said adenoviral vectors and/or said shuttle plasmid vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and (b) harvesting the resultant adenoviral vectors. It is a further object of the present invention to provide for methods of generating a cellular immune response against a protein in an individual comprising administering to the individual an adenovirus vaccine vector comprising a) a replication defective adenoviral vector, at least partially deleted in E1, comprising a wildtype adenovirus cis-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about 450 (preferably, 1-450) and, preferably, 3511-3523 of a wildtype adenovirus sequence, and, b) a gene expression cassette comprising:(i) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (ii) a heterologous promoter operatively linked to the nucleic acid of part a); and (iii) a transcription terminator and/or a 25 polyadenylation site.

It is also an object of the present invention to provide various alternatives for vaccine administration regimes, namely administration of one or more adenoviral and/or DNA plasmid vaccines described herein to provide effective immunoprophylaxis for uninfected individuals or a therapeutic treatment for HIV infected patients. Such processes include but are not limited to multivalent HIV-1 vaccine compositions, various combined modality regimes as well as various prime/boost alternatives. These methods of administration, relating to vaccine composition and/or scheduled administration, will increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

As used throughout the specification and claims, the following definitions and abbreviations are used:

"HAART" refers to - highly active antiretroviral therapy -.

"first generation" vectors are characterized as being replication-defective.

They typically have a deleted or inactivated E1 gene region, and preferably have a deleted or inactivated E3 gene region as well.

"AEX" refers to Anion Exchange chromatography.

"QPA" refers to Quick PCR-based Potency Assay.

"bps" refers to basepairs.

"s" or "str" denotes that the transgene is in the E1 parallel or "straight" orientation.

"PBMCs" refers to peripheral blood monocyte cells.

"FL" refers to full length.

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"FLgag" refers to a full-length optimized gag gene, as shown in Figure 2.

"Ad5-Flgag" refers to an adenovirus serotype 5 replication deficient virus which carries an expression cassette which comprises a full length optimized gag gene under the control of a CMV promoter.

"Promoter" means a recognition site on a DNA strand to which an RNA polymerase binds. The promoter forms an initiation complex with RNA polymerase to initiate and drive transcriptional activity. The complex can be modified by activating sequences such as enhancers or inhibiting sequences such as silencers.

"Leader" means a DNA sequence at the 5' end of a structural gene which is transcribed along with the gene. This usually results a protein having an N-terminal peptide extension, often referred to as a pro-sequences.

"Intron" means a section of DNA occurring in the middle of a gene which does not code for an amino acid in the gene product. The precursor RNA of the intron is excised and is therefore not transcribed into mRNA not translated into protein.

"Immunologically relevant" or "biologically active" means (1) with regards to a viral protein, that the protein is capable, upon administration, of eliciting a measurable immune response within an individual sufficient to retard the propagation and/or spread of the virus and/or to reduce the viral load present within the individual; or (2) with regards to a nucleotide sequence, that the sequence is capable of encoding for a protein capable of the above.

"Cassette" refers to a nucleic acid sequence which is to be expressed, along with its transcription and translational control sequences. By changing the cassette, a vector can express a different sequence.

"bGHpA" refers to the bovine growth hormone transcription terminator/polyadenylation sequence.

"tPAgag" refers to a fusion between the leader sequence of the tissue plasminogen activator leader sequence and an optimized HIV gag gene, as exemplified in Figure 30A-B, whether in a DNA or adenovirus-based vaccine vector.

Where utilized, "IA" or "inact" refers to an <u>inactivated</u> version of a gene (e.g. IApol).

"MCS" is "multiple cloning site".

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In general, adenoviral constructs, gene constructs are named by reference to the genes contained therein. For example:

"Ad5 HIV-1 gag", also referred to as the original HIV-1 gag adenoviral vector, is a vector containing a transgene cassette composed of a hCMV intron A promoter, the full length version of the human codon-optimized HIV-1 gag gene, and the bovine growth hormone polyadenylation signal. The transgene was inserted in the E1 antiparallel orientation in an E1 and E3 deleted adenovector.

"MRK Ad5 HIV-1 gag" also referred to as "MRKAd5gag" or "Ad5gag2" is an adenoviral vector taught herein which is deleted of E1, comprises basepairs 1-450 and 3511-3523, and has a human codon-optimized HIV-1 gene in an E1 parallel orientation under the control of a CMV promoter without intron A. The construct also comprises a bovine growth hormone polyadenylation signal.

"pV1InsHIVgag", also referred to as "HIVFLgagPR9901", is a plasmid comprising the CMV immediate-early (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone.

"pV1JnsCMV(no intron)-FLgag-bGHpA" is a plasmid derived from pV1JnsHIVgag which is deleted of the intron A portion of CMV and which comprises the full length HIV gag gene. This plasmid is also referred to as "pV1JnsHIVgag-bGHpA", pV1Jns-hCMV-FL-gag-bGHpA" and "pV1JnsCMV(no intron) + FLgag + bGHpA".

"pV1JnsCMV(no intron)-FLgag-SPA" is a plasmid of the same composition as pV1JnsCMV(no intron)-FLgag-bGHpA except that the SPA termination sequence replaces that of bGHpA. This plasmid is also referred to as "pV1Jns-HIVgag-SPA" and pV1Jns-hCMV-FLgag-SPA".

"pdelE1sp1A" is a universal shuttle vector with no expression cassette (i.e., no promoter or polyA). The vector comprises wildtype adenovirus serotype 5 (Ad5) sequences from bp 1 to bp 341 and bp 3524 to bp 5798, and has a multiple cloning

site between the Ad5 sequences ending 341 bp and beginning 3524 bp. This plasmid is also referred to as the original Ad 5 shuttle vector.

"MRKpdelE1sp1A" or "MRKpdelE1(Pac/pIX/pack450)" or

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"MRKpdelE1(Pac/pIX/pack450)Cla1" is a universal shuttle vector with no expression cassette (i.e. no promoter or polyA) comprising wildtype adenovirus serotype 5 (Ad5) sequences from bp1 to bp450 and bp 3511 to bp 5798. The vector has a multiple cloning site between the Ad5 sequence ending 450 bp and beginning 3511 bp. This shuttle vector may be used to insert the CMV promoter and the bGHpA fragments in both the straight ("str". or E1 parallel) orientation or in the opposite (opp. or E1 antiparallel) orientation)

"MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.)" is still another shuttle vector which is the modified vector that contains the CMV promoter (no intronA) and the bGHpA fragments. The expression unit containing the hCMV promoter (no intron A) and the bovine growth hormone polyadenylation signal has been inserted into the shuttle vector such that insertion of the gene of choice at a unique *BgI*II site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1/E3+)Cla1 pre-plasmid. This shuttle vector, as shown in Figures 22 and 23, was used to insert the respective IApol and G2A,LLAA nef genes directly into.

"MRKpdelE1-CMV(no intron)-FLgag-bGHpA" is a shuttle comprising Ad5 sequences from basepairs 1-450 and 3511-5798, with an expression cassette containing human CMV without intron A, the full-length human codon-optimized HIV gag gene and bovine growth hormone polyadenylation signal. This plasmid is also referred to as "MRKpdelE1 shuttle +hCMV-FL-gag-BGHpA"

"MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA" is an adenoviral vector comprising all Ad5 sequences except those nucleotides encompassing the E1 region (from 451-3510), a human CMV promoter without intron A, a full-length human codon-optimized HIV gag gene, and a bovine growth hormone polyadenylation signal. This vector is also referred to as "MRKpAdHVE3 + hCMV-FL-gag-BGHpA", "MRKpAd5HIV-1gag", "MRKpAd5gag", "pMRKAd5gag" or "pAd5gag2".

"pV1Jns-HIV-pol inact(opt)" or "pV1Jns-HIV IA pol (opt) is the inactivated Pol gene (contained within SEQ ID NO:3) cloned into the BgIII site of V1Jns (Figure 17A-C). As noted herein, various derivatives of HIV-1 pol may be cloned into a plasmid expression vector such as V1Jns or V1Jns-tPA, thus serving directly as DNA vaccine candidates or as a source for subcloning into an appropriate adenoviral vector.

"MRKpdel+hCMVmin+FL-pol+bGHpA(s)" is the "MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.)" shuttle mentioned above which contains the IA pol gene is the proper orientation. This shuttle vector is used in a bacterial recombination with MRKpAd(E1-/E3+)Cla1.

"MRKpAd+hCMVmin+FL-pol+bGHpA(S)E3+", also referred to herein as "pMRKAd5pol", is the pre-adenovirus plasmid which comprises a CMV-pol inact(opt)-pGHpA construct. The construction of this pre-adenovirus plasmid is shown in Figure 22.

"pV1Jns/nef (G2A,LLAA)" or "V1Jns/opt nef (G2A,LLAA)" comprises codon optimized HTV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175 (SEQ ID NO:13; which comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662). This fragment is subcloned into the Bgl II site of V1Jns and/orV1Jns-tPA (Figures 16A-B). As noted above for HTV-1 pol, HTV-1 nef constructs may be cloned into a plasmid expression vector such as V1Jns or V1Jns-tPA, thus serving directly as DNA vaccine candidates or as a source for subcloning into an appropriate adenoviral vector.

"MRKpdelE1hCMVminFL-nefBGHpA(s)", also referred to herein as "pMRKAd5nef", is the pre-adenovirus plasmid which comprises a CMV-nef (G2A,LLAA) codon optimized sequence. The construction of this pre-adenovirus plasmid is shown in Figure 23.

BRIEF DESCRIPTION OF THE FIGURES

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Figure 1 shows the original HIV-1 gag adenovector (Ad5HIV-1gag). This vector is disclosed in PCT International Application No. PCT/US00/18332 (WO 01/02607) filed July 3, 2000, claiming priority to U.S. Provisional Application Serial No. 60/142,631, filed July 6, 1999 and U.S. Application Serial No. 60/148,981, filed August 13, 1999, all three applications which are hereby incorporated by reference.

Figure 2 shows the nucleic acid sequence (SEQ ID NO: 29) of the optimized human HIV-1 gag open reading frame.

Figure 3 shows diagrammatically the new transgene constructs in comparison with the original gag transgene.

Figure 4 shows the modifications made to the original adenovector backbone in the generation of the novel vectors of the instant invention.

Figure 5 shows the virus mixing experiments that were carried out to determine the effects of the addition made to the packaging signal region (Expt. #1) and the E3 gene on viral growth (Expt. #2). The bars denote the region of modifications made to the E1 deletion.

Figure 6 shows an autoradiograph of viral DNA analysis following the viral mixing experiments described in Examples 6 and 7.

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Figures 7A, 7B and 7C are as follows: Figure 7A shows the hCMV-Flgag-bGHpA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Both E1 parallel and E1 antiparallel transgene orientation are represented. Figure 7B shows the hCMV-Flgag-SPA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Again, both E1 parallel and E1 antiparallel transgene orientation are represented. Figure 7C shows the mCMV-Flgag-bGHpA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Once again, both E1 parallel and E1 antiparallel transgene orientation are represented.

Figure 8A shows the experiment designed to test the effect of transgene orientation.

Figure 8B shows the experiments designed to test the effect of polyadenylation signal.

Figure 9 shows viral DNA from the four adenoviral vectors tested (Example 12) at P5, following *Bst*E11 digestion.

Figure 10 shows viral DNA analysis of passages 11 and 12 of MRKpAdHVE3, MRKAd5HIV-1gag, and MRKAd5HIV-1gagE3-.

Figure 11 shows viral DNA analysis (*Hind*III digestion) of passage 6 MRKpAdHVE3 and MRKAd5HIV-1gag used to initiate the viral competition study. The last two lanes are passage 11 analysis of duplicate passages of the competition study (each virus at MOI of 280 viral particles).

Figure 12 shows viral DNA analysis by *Hind* III digestion on high passage numbers for MRKAd5HIV-1gag in serum-containing media with collections made at specified times. The first lane shows the 1kb DNA size marker. The other lanes represent pre-plasmid control (digested with Pac1 and *Hind*III), MRKAd5HIV-1gag at P16, P19, and P21.

Figure 13 shows serum anti-p24 levels at 3 wks post i.m. immunization of balb/c mice (n=10) with varying doses of several Adgag constructs: (A) MRK Ad5 HIV-1 gag (through passage 5); (B) MRKAd5 hCMV-FLgag-bGHpA (E3-); (C) MRKAd5 hCMV-FLgag-SPA (E3+); (D) MRKAd5 mCMV-FLgag-bGHpA (E3+);

(E) research lot (293 cell-derived) of Ad5HIV-1 gag; and (F) clinical lot (Ad5gagFN0001) of Ad5HIV-1 gag. Reported are the geometric mean titers (GMT) for each cohort along with the standard error bars.

Figure 14 shows a restriction map of the pMRKAd5HIV-1gag vector.

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Figures 15A-X illustrates the nucleotide sequence of the pMRKAd5HIV-1gag vector (SEQ ID NO:27.[coding] and SEQ ID NO:28 [non-coding]).

Figures 16A-B shows a schematic representation of DNA vaccine expression vectors V1Jns (A) and V1Jns-tPA (B), which are utilized for HIV-1 gag, pol and nef constructs in various DNA/viral vector combined modality regimens as disclosed herein.

Figures 17A-C shows the nucleotide (SEQ ID NO:3) and amino acid sequence (SEQ ID NO:4) of IA-Pol. Underlined codons and amino acids denote mutations, as listed in Table 1.

Figure 18 shows codon optimized nucleotide and amino acid sequences through the fusion junction of tPA-pol inact(opt) (contained within SEQ ID NOs: 7 and 8, respectively). The underlined portion represents the NH₂-terminal region of IA-Pol.

Figures 19A-B show a nucleotide sequence comparison between wild type nef(jrfl) and codon optimized nef. The wild type nef gene from the jrfl isolate consists of 648 nucleotides capable of encoding a 216 amino acid polypeptide. WT, wild type sequence (SEQ ID NO:19); opt, codon-optimized sequence (contained within SEQ ID NO:1). The Nef amino acid sequence is shown in one-letter code (SEQ ID NO:2).

Figures 20A-C show nucleotide sequences at junctions between nef coding sequence and plasmid backbone of nef expression vectors V1Jns/nef (Figure 20A), V1Jns/nef(G2A,LLAA) (Figure 20B), V1Jns/tpanef (Figure 20C) and V1Jns/tpanef(LLAA) (Figure 20C, also). 5' and 3' flanking sequences of codon optimized nef or codon optimized nef mutant genes are indicated by bold/italic letters; nef and nef mutant coding sequences are indicated by plain letters. Also indicated (as underlined) are the restriction endonuclease sites involved in construction of respective nef expression vectors. V1Jns/tpanef and V1Jns/tpanef(LLAA) have identical sequences at the junctions.

Figure 21 shows a schematic presentation of nef and nef derivatives. Amino acid residues involved in Nef derivatives are presented. Glycine 2 and Leucine174 and 175 are the sites involved in myristylation and dileucine motif, respectively. For both versions of the tpanef fusion genes, the putative leader peptide cleavage sites are

indicated with "*", and a exogenous serine residue introduced during the construction of the mutants is underlined.

Figure 22 shows diagrammatically the construction of the pre-adenovirus plasmid construct, MRKAd5Pol.

Figure 23 shows diagrammatically the construction of the pre-adenovirus plasmid construct, MRKAd5Nef.

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Figure 24 shows a comparison of clade B vs. clade C anti-gag T cell responses in clade B HIV-infected subjects.

Figure 25 shows a comparison of clade B vs. clade C anti-nef T cell responses in clade B HIV-infected subjects.

Figures 26A-AO illustrates the nucleotide sequence of the pMRKAd5HIV-1pol adenoviral vector (SEQ ID NO:32 [coding] and SEQ ID NO:33 [non-coding]), comprising the coding region of the inactivated pol gene (SEQ ID NO3).

Figures 27A-AM illustrates the nucleotide sequence of the pMRKAd5HIV-1 nef adenoviral vector (SEQ ID NO:34 [coding] and SEQ ID NO:35 [non-coding]), comprising the coding region of the inactivated pol gene (SEQ ID NO13).

Figure 28 shows the stability of MRKAd5 vectors comprising various promoter fragments (hCMV or mCMV) and terminations signals (bGH or SPA) in E3(+) or E3(-) backbones.

Figures 29A and B shows the anion-exchange HPLC viral particle concentrations of the freeze-thaw recovered cell associated virus at the 24, 36, 48, and 60 hpi time points (Figure 29A) and the timcourse QPA supernatant titers (Figure 29B) for MRKAd5gag, MRKAd5pol and MRKAd5nef.

Figure 30 shows the nucleotide sequence (SEQ ID NO:36) and amino acid sequence (SEQ ID NO:37) comprising the open reading frame of a representative tPA-gag fusion for use in the DNA and/or adenoviral vaccine disclosed herein.

Figure 31 shows the intracellular γIFN staining of PBMCs collected at week 10 (post DNA prime) and week 30 (post Ad boost). The cells were stimulated overnight in the presence or absence of the gag peptide pool. They were subsequently stained using fluorescence-tagged anti-CD3, anti-CD8, anti-CD4, and anti-γIFN monoclonal antibodies. Each plot shows all CD3+ T cells which were segregated in terms of positive staining for surface CD8 and γIFN production. The numbers in the upper right and lower right quadrants of each plot are the percentages of CD3⁺ cells that were CD8⁺γIFN⁺ and CD4⁺γIFN⁺, respectively.

Figure 32 shows a comparison of single-modality adenovirus immunization with DNA + adjuvant prime/adenovirus boost immunization.

Figures 33A-B show the nucleotide sequence (SEQ ID NO: 38) of the open reading frame for the gag-IApol fusion of Example 29.

Figures 34A-B show the protein sequence (SEQ ID NO:39) of the gag-IApol fustion frame.

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DETAILED DESCRIPTION OF THE INVENTION

A novel replication-defective, or "first generation," adenoviral vector suitable for use in gene therapy or nucleotide-based vaccine vectors is described. This vector is at least partially deleted in E1 and comprises a wildtype adenovirus cis-acting packaging region from about base pair 1 to between about base pair 342 (more preferably, 400) to about 458 (preferably, 1-450) and, preferably, 3511-3523 of a wild-type adenovirus sequence. It has been found that a vector of this description possesses enhanced growth characteristics, with approximately 5-10 fold greater amplification rates, and is more potent allowing lower doses of virus to be used to generate equivalent immunity. The vector, furthermore, generates a harvested recombinant adenovirus which shows greater cellular-mediated immune responses than replication-deficient vectors not comprising this region (basepairs 342-450). Adenoviral constructs derived from these vectors are, further, very stable genetically, particularly those comprising a transgene under the control of a hCMV promoter devoid of intron A. Viruses in accordance with this description were passaged continually and analyzed; see Example 12. Each virus analyzed maintained it correct genetic structure. Analysis was also carried out under propagation conditions similar to that performed in large scale production. Again, the vectors were found to possess enhanced genetic stability; see Figure 12. Following 21 passages, the viral DNA showed no evidence of rearrangement, and was highly reproducible from one production lot to the next. The outcome of all relevant tests indicate that the adenoviral vector is extremely well suited for large-scale production of recombinant, replication-deficient adenovirus, as shown herein with the data associated with Figure 28.

A preferred adenoviral vector in accordance with this description is a vector comprising basepairs 1-450, which is deleted in E3. This vector can accommodate up to approximately 7,500 base pairs of foreign DNA inserts (or exogenous genetic material). Another preferred vector is one retaining E3 which comprises basepairs 1-450. A preferred vector of this description is an E3+ vector comprising basepairs 1-450 and 3511-3523. This vector, when deleted of the region spanning basepairs 451-3510, can accommodate up to approximately, 4,850 base pairs of foreign DNA inserts

(or exogenous genetic material). The cloning capacities of the above vectors have been determined using 105% of the wildtype Ad5 sequence as the upper genome size limit.

Wildtype adenovirus serotype 5 is used as the basis for the specific basepair numbers provided throughout the specification. The wildtype adenovirus serotype 5 sequence is known and described in the art; see, Chroboczek et al., 1992 J. Virology 186:280, which is hereby incorporated by reference. Accordingly, a particular embodiment of the instant invention is a vector based on the adenovirus serotype 5 sequence. One of skill in the art can readily identify the above regions in other adenovirus serotypes (e.g., serotypes 2, 4, 6, 12, 16, 17, 24, 31, 33, and 42), regions defined by basepairs corresponding to the above basepair positions given for adenovirus serotype 5. Accordingly, the instant invention encompasses all adenoviral vectors partially deleted in E1 comprising basepairs corresponding to 1-450 (particularly, 342-450) and, preferably, 3511-3523 of a wild-type adenovirus serotype 5 (Ad5) nucleic acid sequence. Particularly preferred embodiments of the instant invention are those derived from adenoviruses like Ad5 which are classified in subgroup C (e.g., Ad2).

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Vectors in accordance with the instant invention are at least partially deleted in E1. Preferably the E1 region is completely deleted or inactivated. Most preferably, the region deleted of E1 is within basepairs 451-3510. It is to be noted that the extended 5' and 3' regions of the disclosed vectors are believed to effectively reduce the size of the E1 deletion of previous constructs without overlapping any part of the E1A/E1B gene present in the cell line used, i.e., the PER.C6® cell line transfected with base pairs 459-3510. Overlap of adenoviral sequences is avoided because of the possibility of recombination. One of ordinary skill in the art can certainly appreciate that the instant invention can, therefore, be modified if a different cell line transfected with a different segment of adenovirus DNA is utilized. For purposes of exemplification, a 5' region of base pairs 1 to up to 449 is more appropriate if a cell line is transfected with adenoviral sequence from base pairs 450-3510. This holds true as well in the consideration of segments 3' to the E1 deletion.

Preferred embodiments of the instant invention possess an intact E3 region (i.e., an E3 gene capable of encoding a functional E3). Alternate embodiments have a partially deleted E3, an inactivated E3 region, or a sequence completely deleted of E3. Applicants have found, in accordance with the instant invention, that virus comprising the E3 gene were able to amplify more rapidly compared with virus not comprising an E3 gene; see Figure 6 wherein a diagnostic CsCl band corresponding to the E3+ virus

tested (5,665 bp) was present in greater amount compared with the diagnostic band of 3,010 bp corresponding to the E3- virus. These results were obtained following a virus competition study involving mixing equal MOI ratio (1:1) of adenovectors both comprising the E3 gene and not comprising the E3 gene. This increased amplification capacity of the E3+ adenovectors was subsequently confirmed with growth studies; see Table 4A, wherein the E3+ virus exhibit amplification ratios of 470, 420 and 320 as compared with the 115 and 40-50 of the E3- constructs.

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As stated above, vectors in accordance with the instant invention can accommodate up to approximately 4,850 base pairs of exogenous genetic material for an E3+ vector and approximately 7,500 base pairs for an E3- vector. Preferably, the insert brings the adenoviral vector as close as possible to a wild-type genomic size (e.g., for Ad5, 35,935 basepairs). It is well known that adenovirus amplifies best when they are close to their wild-type genomic size.

The genetic material can be inserted in an E1-parallel or an E1 anti-parallel orientation, as such is illustrated in Figure 7A, 7B, 7C and Figure 8A. Particularly preferred embodiments of the instant invention, have the insert in an E1-parallel orientation. Applicants have found, via competition experiments with plasmids containing transgenes in differing orientation (Figure 8A), that vector constructs with the foreign DNA insert in an E1-parallel orientation amplify better and actually outcompete E1-antiparallel-oriented transgenes. Viral DNA analysis of the mixtures at passage 3 and certainly at passage 6, showed a greater ratio of the virus carrying the transgene in the E1 parallel orientation as compared with the E1 anti-parallel version. By passage 10, the only viral species observed was the adenovector with the transgene in the E1 parallel orientation for both transgenes tested.

Adenoviral vectors in accordance with the instant invention are particularly well suited to effectuate expression of desired proteins, one example of which is an HIV protein, particularly an HIV full length gag protein. Exogenous genetic material encoding a protein of interest can exist in the form of an expression cassette. A gene expression cassette preferably comprises (a) a nucleic acid encoding a protein of interest; (b) a heterologous promoter operatively linked to the nucleic acid encoding the protein; and (c) a transcription terminator.

The transcriptional promoter is preferably recognized by an eukaryotic RNA polymerase. In a preferred embodiment, the promoter is a "strong" or "efficient" promoter. An example of a strong promoter is the immediate early human cytomegalovirus promoter (Chapman et al, 1991 *Nucl. Acids Res*19:3979-3986, which is incorporated by reference), preferably without intronic sequences. Most preferred

for use within the instant adenoviral vector is a human CMV promoter without intronic sequences, like intron A. Applicants have found that intron A, a portion of the human cytomegalovirus promoter (hCMV), constitutes a region of instability for adenoviral vectors. CMV without intron A has been found to effectuate (Examples 1-3) comparable expression capabilities in vitro when driving HIV gag expression and, furthermore, behaved equivalently to intron A-containing constructs in Balb/c mice in vivo with respect to their antibody and T-cell responses at both dosages of plasmid DNA tested (20 µg and 200 µg). Those skilled in the art will appreciate that any of a number of other known promoters, such as the strong immunoglobulin, or other eukaryotic gene promoters may also be used, including the EF1 alpha promoter, the murine CMV promoter, Rous sarcoma virus (RSV) promoter, SV40 early/late promoters and the beta-actin promoter.

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In preferred embodiments, the promoter may also comprise a regulatable sequence such as the Tet operator sequence. This would be extremely useful, for example, in cases where the gene products are effecting a result other than that desired and repression is sought.

The combination of the CMV promoter (devoid of the intron A region) with the BGH terminator is particularly preferred although other promoter/terminator combinations in the context of FG adenovirus may also be used.

Other embodiments incorporate a leader or signal peptide into the transgene. A preferred leader is that from the tissue-specific plasminogen activator protein, tPA. Examples include but are not limited to the various tPA-gag, tPA-pol and tPA-nef adenovirus-based vaccines disclosed throughout this specification.

In view of the improved adenovirus vectors described herein, an essential portion of the present invention are adenoviral-based HIV vaccines comprising said adenovirus backbones which may be administered to a mammalian host, preferably a human host, in either a prophylactic or therapeutic setting. The HIV vaccines of the present invention, whether administered alone or in combination regimens with other viral- or non-viral-based DNA vaccines, should elicit potent and broad cellular immune responses against HIV that will either lessen the likelihood of persistent virus infection and/or lead to the establishment of a clinically significant lowered virus load

subject to HIV infection or in combination with HAART therapy, mitigate the effects of previously established HIV infection (antiviral immunotherapy(ARI)). While any HIV antigen (e.g., gag, pol, nef, gp160, gp41, gp120, tat, rev, etc.) may be utilized in the herein described recombinant adenoviral vectors, preferred embodiments include the codon optimized p55 gag antigen (herein exemplified as MRKAd5gag), pol and nef. Sequences based on different Clades of HIV-1 are suitable for use in the instant invention, most preferred of which are Clade B and Clade C. Particularly preferred embodiments are those sequences (especially, codon-optimized sequences) based on concensus Clade B sequences. Preferred versions of the MRKAd5pol and MRKAd5nef series of adenoviral vaccines will encode modified versions of pol or nef, as discussed herein. Preferred embodiments of the MRKAd5HIV-1 vectors carrying HIV envelope genes and modifications thereof comprise the HIV codon-optimized *env* sequences of PCT International Applications PCT/US97/02294 and PCT/US97/10517, published August 28, 1997 (WO 97/31115) and December 24, 1997, respectively; both documents of which are hereby incorporated by reference.

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A most preferred aspect of the instant invention is the disclosed use of the adenoviral vector described above to effectuate expression of HIV gag. Sequences for many genes of many HIV strains are publicly available in GENBANK and primary, field isolates of HIV are available from the National Institute of Allergy and Infectious Diseases (NIAID) which has contracted with Quality Biological (Gaithersburg, MD) to make these strains available. Strains are also available from the World Health Organization (WHO), Geneva Switzerland. It is preferred that the gag gene be from an HIV-1 strain (CAM-1; Myers et al, eds. "Human Retroviruses and AIDS: 1995, IIA3-IIA19, which is hereby incorporated by reference). This gene closely resembles the consensus amino acid sequence for the clade B (North American/European) sequence. Therefore, it is within the purview of the skilled artisan to choose an appropriate nucleotide sequence which encodes a specific HIV gag antigen, or immunologically relevant portion thereof. As shown in Example 25, a clade B or clade C based p55 gag antigen will potentially be useful on a global scale. As noted herein, the transgene of choice for insertion in to a DNA or MRKAd-based adenoviral vector of the present invention is a codon optimized version of p55 gag. Such a MRKAd5gag adenoviral vector is documented in Example 11 and is at least referred to herein as MRKAd5HIV-1gag. Of course, additional versions are contemplated, including but not limited to modifications such as promoter (e.g., mCMV for hCMV) and/or pA-terminations signal (SPA for bGH) switching, as well as generating MRK Ad5 backbones with or without deletion of the Ad5 E3 gene.

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The present invention also relates a series of MRKAd5pol-based adenoviral vaccines which are shown herein to generate cellular immune responses subsequent to administration in mice and non-human primate studies. Several of the MRKAd5pol series are exemplified herein. One such adenoviral vector is referred to as MRKAd5hCMV-inact opt pol(E3+), which comprises the MRKAd5 backbone, the hCMV promoter (no intron A), an inactivated pol transgene, and contains the Ad5 E3 gene in the adenoviral backbone. A second exemplified pre-adenovirus plasmid and concomitant virus is referred to as MRKAd5hCMV-inact opt pol(E3-), which is identical to the former adenoviral vector except that the E3 is deleted. Both 10 constructions contain a codon optimized, inactivated version of HIV-1 Pol, wherein at least the entire coding region is disclosed herein as SEQ ID NO:3 and the expressed protein is shown as SEQ ID NO:4 (see also Figure 17A-C and Table 1, which show targeted deletion for inactivated pol. This and other preferred codon optimized versions of HIV Pol as disclosed herein are essentially as described in U.S. Application Serial No. 09/745,221, filed December 21, 2000 and PCT International Application PCT/US00/34724, also filed December 21, 2000, both documents which are hereby incorporated by reference. As disclosed in the above-mentioned documents, the open reading frame for these codon-optimized HIV-1 Pol-based DNA vaccines are represented by codon optimized DNA molecules encoding codon optimized HIV-1 Pol (e.g. SEQ ID NO:2), codon optimized HIV-1 Pol fused to an amino terminal localized leader sequence (e.g. SEQ ID NO:6), and especially preferable, and exemplified by the MRKAd5-Pol construct in e.g., Example 19. biologically inactivated pol ("inact opt Pol"; e.g., SEQ ID NO:4) which is devoid of significant PR, RT, RNase or IN activity associated with wild type Pol. In addition, a construct related to SEQ ID NO:4 is contemplated which contains a leader peptide at the amino terminal region of the IA Pol protein. A specific construct is ligated within an appropriate DNA plasmid vector containing regulatory regions operatively linked to the respective HIV-1 Pol coding region, with or without a nucleotide sequence encoding a functional leader peptide. To this end, various HIV-1 Pol constructs disclosed herein relate to open reading frames for cloning to the enhanced first generation Ad vectors of the present invention (such a series of MRKAd5pol adenoviral vaccine vectors), including but not limited to wild type Pol (comprising the DNA molecule encoding WT opt Pol, as set forth in SEQ ID NO:2), tPA-opt WTPol, (comprising the DNA molecule encoding tPA Pol, as set forth in SEQ ID NO:6), inact opt Pol (comprising the DNA molecule encoding IA Pol, as set forth in SEQ ID NO:4), and tPA-inact opt Pol, (comprising the DNA molecule encoding tPA-inact opt

Pol, as set forth in SEQ ID NO:8). The pol-based versions of enhanced first generation adenovirus vaccines elicit CTL and Th cellular immune responses upon administration to the host, including primates and especially humans. As noted in the above, an effect of the cellular immune-directed vaccines of the present invention should be a lower transmission rate to previously uninfected individuals and/or reduction in the levels of the viral loads within an infected individual, so as to prolong the asymptomatic phase of HIV-1 infection.

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The present invention further relates to a series of MRKAd5nef-based adenoviral vaccines which, similar to HIV gag and pol antigens, generate cellular immune responses subsequent to administration in mice and non-human primate studies. The MRKAd5nef series are exemplified herein by utilizing the improved MRK adenoviral backbone in combination with modified versions of HIV nef. These exemplified MRKAd5nef vectors are as follows: (1) MRKAd5hCMVnef(G2A,LLAA) (E3+), which comprises the improved MRKAd5 backbone, a human CMV promoter an intact Ad5 E3 gene and a modified nef gene: (2) MRKAd5mCMVnef(G2A,LLAA) (E3+), which is the same as (1) above but substituting a murine CMV promoter for a human CMV promoter; and (3) MRKAd5mCMV-tpanef(LLAA) (E3+), which is the same as (2) except that the nef transgene is tpanef(LLAA). Codon optimized versions of HIV-1 Nef and HIV-1 Nef modifications are essentially as described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference. Particular embodiments of codon optimized Nef and Nef modifications relate to a DNA molecule encoding HIV-1 Nef from the HIV-1 ifrl isolate wherein the codons are optimized for expression in a mammalian system such as a human. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:9, while the expressed open reading frame is disclosed herein as SEQ ID NO:10. Another embodiment of Nef-based coding regions for use in the adenoviral vectors of the present invention comprise a codon optimized DNA molecule encoding a protein containing the human plasminogen activator (tpa) leader peptide fused with the NH2-terminus of the HIV-1 Nef polypeptide. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:11, while the expressed open reading frame is disclosed herein as SEQ ID NO:12. Another modified Nef optimized coding region relates to a DNA molecule encoding optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175, herein

described as opt nef (G2A, LLAA). The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:13, while the expressed open reading frame is disclosed herein as SEQ ID NO:14. MRKAd5nef vectors (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+) and (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+) contain this transgene. An additional embodiment relates to a DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA), comprises an open reading frame which encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175, herein referred to as opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, while the expressed open reading frame is disclosed herein as SEQ ID NO:16. The MRKAd5nef vector "MRKAd5mCMV-tpanef(LLAA) (E3+)" contains this transgene.

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Along with the improved MRKAd5gag adenovirus vaccine vector described herein, generation of a MRKAd5pol and MRKAd5nef adenovirus vector provide for enhanced HIV vaccine capabilities. Namely, the generation of this trio of adenoviral vaccine vectors, all shown to generate effective cellular immune responses subsequent to host administration, provide for the ability to administer these vaccine candidates not only alone, but preferably as part of a divalent (i.e., gag and nef, gag and pol, or pol and nef components) or a trivalent vaccine (i.e., gag, pol and nef components). Therefore, a preferred aspect of the present invention are vaccine formulations and associated methods of administration and concomitant generation of host cellular immune responses associated with formulating three separate series of MRKAd5based adenoviral vector vaccines. Of course, this MRKAd5 vaccine series based on distinct HIV antigens promotes expanded opportunities for formulation of a divalent or trivalent vaccine, or possibly administration of separate formulations of one or more monovalent or divalent formulations within a reasonable window of time. It is also within the scope of the present invention to embark on combined modality regimes which include multiple but distinct components from a specific antigen. An example, but certainly not a limitation, would be separate MRKAd5pol vectors, with one vaccine vector expressing wild type Pol (SEQ ID NO:2) and another MRKAd5pol vector expressing inactivated Pol (SEQ ID NO:6). Another example might be separate MRKAd5nef vectors, with one vaccine vector expressing the tPA/LLAA version of Nef (SEQ ID NO:16) and another MRKAd5nef vector expressing the G2A,LLAA modified version of Nef (SEQ ID NO:14). Therefore, the MRKAd5 adenoviral vectors of the present invention may be used in combination

with multiple, distinct HIV antigen classes. Each HIV antigen class is subject to sequence manipulation, thus providing for a multitude of potential vaccine combinations; and such combinations are within the scope of the present invention. The utilization of such combined modalities vaccine formulation and administration increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

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The present invention also relates to application of a mono-, dual-, or trimodality administration regime of the MRKAd5gag, pol and nef adenoviral vaccine series in a prime/boost vaccination schedule. This prime/boost schedule may include any reasonable combination of the MRKAd5gag, pol and nef adenoviral vaccine series disclosed herein. In addition, a prime/boost regime may also involve other viral and/or non-viral DNA vaccines. A preferable addition to an adenoviral vaccine vector regime includes but is not limited to plasmid DNA vaccines, especially DNA plasmid vaccines that contain at least one of the codon optimized gag, pol and nef constructions, as disclosed herein.

Therefore, one aspect of this invention is the administration of the adenoviral vector containing the optimized gag gene in a prime/boost regiment in conjunction with a plasmid DNA encoding gag. To distinguish this plasmid from the adenoviralcontaining shuttle plasmids used in the construction of an adenovirus vector, this plasmid will be referred to as a "vaccine plasmid" or "DNA plasmid vaccine". Preferred vaccine plasmids for use in this administration protocol are disclosed in pending U.S. patent application 09/017,981, filed February 3, 1998 and WO98/34640, published August 13, 1998, both of which are hereby incorporated by reference. Briefly, the preferred vaccine plasmid is designated V1Jns-FLgag, which expresses the same codon-optimized gag gene as the adenoviral vectors of this invention (see Figure 2 for the nucleotide sequence of the exemplified optimized codon version of full length p55 gag). The vaccine plasmid backbone, designated V1Jns contains the CMV immediate-early (IE) promoter and intron A, a bovine growth hormone-derived polyadenylation and transcription termination sequence as the gene expression regulatory elements, and a minimal pUC backbone; see Montgomery et al., 1993, DNA Cell Biol. 12:777-783. The pUC sequence permits high levels of plasmid production in E. coli and has a neomycin resistance gene in place of an ampicillin resistance gene to provide selected growth in the presence of kanamycin. Alternatively, a vaccine plasmid which has the CMV promoter deleted of intron A can be used. Those of skill in the art will recognize that alternative vaccine plasmid

vectors may be easily substituted for these specific constructs, and this invention specifically envisions use of such alternative plasmid DNA vaccine vectors.

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Another aspect of the present invention is a prime/boost regimen which includes a vaccine plasmid which encodes an HIV pol antigen, preferably a codon optimized form of pol and also preferably a vaccine plasmid which comprises a nucleotide sequence which encodes a Pol antigen selected from the group of Pol antigens as shown in SEQ ID NOs: 2, 4, 6 and 8. The variety of potential DNA plasmid vaccines which encode various biologically active forms of HIV-1 Pol. wherein administration, intracellular delivery and expression of the HIV-1 Pol gene of interest elicits a host CTL and Th response. The preferred synthetic DNA molecules of the present invention encode codon optimized wild type Pol (without Pro activity) and various codon optimized inactivated HIV-1 Pol proteins. The HIV-1 pol open reading disclosed herein are especially preferred for pharmaceutical uses, especially for human administration as delivered via a recombinant adenoviral vaccine. especially an enhanced first generation recombinant adenoviral vaccine as described herein. Several embodiments of this portion of the invention are provided in detail below, namely DNA molecules which comprise a HIV-1 pol open reading frame, whether encoding full length pol or a modification or fusion as described herein, wherein the codon usage has been optimized for expression in a mammal, especially a human. Again, these DNA sequences are positioned appropriately within a recombinant adenoviral vector, such as the exemplified recombinant adenoviral vector described herein, so as to promote expression of the respective HIV-1 Pol gene of interest, and subsequent to administration, elicit a host CTL and Th response. Again, these preferred, but in no way limiting, pol genes are as disclosed herein and essentially as described in U.S. Application Serial No. 09/745,221, filed December 21, 2000 and PCT International Application PCT/US00/34724, also filed December 21, 2000, both documents which are hereby incorporated by reference.

A third series of vaccine plasmids which are useful in a combined modality and/or prime/boost regimen are vaccine plasmids which encode an HIV nef antigen or biologically and/or immunologically relevant modification thereof. As noted elsewhere, preferred vaccine plasmids contain a codon optimized form of nef and also preferably comprise a nucleotide sequence which encodes a Nef antigen selected from the group of Nef antigens as shown in SEQ ID NOs: 10, 12, 14 and 16. These preferred nef coding regions are disclosed herein, as well as being described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International

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Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference.

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Therefore, the adenoviral vaccines and plasmid DNA vaccines of this invention may be administered alone, or may be part of a prime and boost administration regimen. A mixed modality priming and booster inoculation scheme will result in an enhanced immune response, particularly is pre-existing anti-vector immune responses are present. This one aspect of this invention is a method of priming a subject with the plasmid vaccine by administering the plasmid vaccine at least one time, allowing a predetermined length of time to pass, and then boosting by administering the adenoviral vaccine. Multiple primings typically, 1-4, are usually employed, although more may be used. The length of time between priming and boost may typically vary from about four months to a year, but other time frames may be used. In experiments with rhesus monkeys, the animals were primed four times with plasmid vaccines, then were boosted 4 months later with the adenoviral vaccine. Their cellular immune response was notably higher than that of animals which had only received adenoviral vaccine. The use of a priming regimen may be particularly preferred in situations where a person has a pre-existing anti-adenovirus immune response.

Furthermore and in the alternative, multiple HIV-1 viral antigens, such as the MRKAd5 adenoviral vaccines disclosed herein, may be ligated into a proper shuttle plasmid for generation of a pre-adenoviral plasmid comprising multiple open reading frames. For example a trivalent vector may comprise a gag-pol-nef fusion, in either a E3(-) or E3(+) background, preferably a E3 deleted backbone, or possible a "2+1" divalent vaccine, such as a gag-pol fusion (i.e., codon optimized p55 gag and inactivated optimized pol; Example 29 and Table 25) within the same MRKAd5 backbone, with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES), as disclosed in International Publication No. WO 95/24485, which is hereby incorporated by reference. Figure 9 shows that the use of multiple promoters and termination sequences provide for similar growth properties, while Figure 28 shows that these MRKAd5gag-based vectors are also stable at least through passage 21. In the absence of the use of IRES-based technology, it is preferred that a distinct promoter be used to support each respective open reading frame, so as to best preserve vector stability. As examples, and certainly not as limitations, potential multiple transgene vaccines may

include a three transgene vector such as hCMV-gagpol-bGHpA + mCMV-nef-SPA in an E3 deleted backbone or hCMV-gagpol-bGHpA + mCMV-nef-SPA(E3+). Potential "2+1" divalent vaccines of the present invention might be a hCMV-gagbGHpA + mCMV-nef-SPA in an E3+ backbone (vector #1) in combination with hCMV-pol-bGHpA in an E3+ backbone (vector #2), with all transgenes in the E1 parallel orientation. Fusion constructs other than the gag-pol fusion described above are also suitable for use in various divalent vaccine strategies and can be composed of any two HIV antigens fused to one another (e.g., nef-pol and gag-nef). These adenoviral compositions are, as above, preferably delivered along with an adenoviral composition comprising an additional HIV antigen in order to diversify the immune response generated upon administration. Therefore, a multivalent vaccine delivered in a single, or possible second, adenoviral vector is certainly contemplated as part of the present invention. Again, this mode of administration is another example of whereby an efficaceous adenovirus-based HIV-1 vaccine may be administered via a combined modality regime. It is important to note, however, that in terms of deciding on an insert for the disclosed adenoviral vectors, due consideration must be dedicated to the effective packaging limitations of the adenovirus vehicle. Adenovirus has been shown to exhibit an upper cloning capacity limit of approximately 105% of the wildtype Ad5 sequence.

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Regardless of the gene chosen for expression, it is preferred that the sequence be "optimized" for expression in a human cellular environment. A "triplet" codon of four possible nucleotide bases can exist in 64 variant forms. That these forms provide the message for only 20 different amino acids (as well as transcription initiation and termination) means that some amino acids can be coded for by more than one codon. Indeed, some amino acids have as many as six "redundant", alternative codons while some others have a single, required codon. For reasons not completely understood. alternative codons are not at all uniformly present in the endogenous DNA of differing types of cells and there appears to exist variable natural hierarchy or "preference" for certain codons in certain types of cells. As one example, the amino acid leucine is specified by any of six DNA codons including CTA, CTC, CTG, CTT, TTA, and TTG (which correspond, respectively, to the mRNA codons, CUA, CUC, CUG, CUU, UUA and UUG). Exhaustive analysis of genome codon frequencies for microorganisms has revealed endogenous DNA of E. coli most commonly contains the CTG leucine-specifying codon, while the DNA of yeasts and slime molds most commonly includes a TTA leucine-specifying codon. In view of this hierarchy, it is generally held that the likelihood of obtaining high levels of expression of a leucine-

rich polypeptide by an *E. coli* host will depend to some extent on the frequency of codon use. For example, a gene rich in TTA codons will in all probability be poorly expressed in *E. coli*, whereas a CTG rich gene will probably highly express the polypeptide. Similarly, when yeast cells are the projected transformation host cells for expression of a leucine-rich polypeptide, a preferred codon for use in an inserted DNA would be TTA.

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The implications of codon preference phenomena on recombinant DNA techniques are manifest, and the phenomenon may serve to explain many prior failures to achieve high expression levels of exogenous genes in successfully transformed host organisms—a less "preferred" codon may be repeatedly present in the inserted gene and the host cell machinery for expression may not operate as efficiently. This phenomenon suggests that synthetic genes which have been designed to include a projected host cell's preferred codons provide a preferred form of foreign genetic material for practice of recombinant DNA techniques. Thus, one aspect of this invention is an adenovirus vector or adenovirus vector in some combination with a vaccine plasmid where both specifically include a gene which is codon optimized for expression in a human cellular environment. As noted herein, a preferred gene for use in the instant invention is a codon-optimized HTV gene and, particularly, HTV gag, pol or nef.

Adenoviral vectors in accordance with the instant invention can be constructed using known techniques, such as those reviewed in Hitt et al, 1997 "Human Adenovirus Vectors for Gene Transfer into Mammalian Cells" Advances in Pharmacology 40:137-206, which is hereby incorporated by reference.

In constructing the adenoviral vectors of this invention, it is often convenient to insert them into a plasmid or shuttle vector. These techniques are known and described in Hitt et al., *supra*. This invention specifically includes both the adenovirus and the adenovirus when inserted into a shuttle plasmid.

Preferred shuttle vectors contain an adenoviral portion and a plasmid portion. The adenoviral portion is essentially the same as the adenovirus vector discussed supra, containing adenoviral sequences (with non-functional or deleted E1 and E3 regions) and the gene expression cassette, flanked by convenient restriction sites. The plasmid portion of the shuttle vector often contains an antibiotic resistance marker under transcriptional control of a prokaryotic promoter so that expression of the antibiotic does not occur in eukaryotic cells. Ampicillin resistance genes, neomycin resistance genes and other pharmaceutically acceptable antibiotic resistance markers may be used. To aid in the high level production of the polynucleotide by

fermentation in prokaryotic organisms, it is advantageous for the shuttle vector to contain a prokaryotic origin of replication and be of high copy number. A number of commercially available prokaryotic cloning vectors provide these benefits. It is desirable to remove non-essential DNA sequences. It is also desirable that the vectors not be able to replicate in eukaryotic cells. This minimizes the risk of integration of polynucleotide vaccine sequences into the recipients' genome. Tissue-specific promoters or enhancers may be used whenever it is desirable to limit expression of the polynucleotide to a particular tissue type.

In one embodiment of this invention, the pre-plasmids (e.g., pMRKAd5pol, pMRKAd5nef and pMRKAd5gag were generated by homologous recombination using the MRKHVE3 (and MRKHVO for the E3- version) backbones and the appropriate shuttle vector, as shown for pMRKAd5pol in Figure 22 and for pMRKAd5nef in Figure 23. The plasmid in linear form is capable of replication after entering the PER.C6[®] cells and virus is produced. The infected cells and media were harvested after viral replication was complete.

Viral vectors can be propagated in various E1 complementing cell lines, including the known cell lines 293 and PER.C6[®]. Both these cell lines express the adenoviral E1 gene product. PER.C6[®] is described in WO 97/00326 (published January 3, 1997) and issued U.S. Patent No. 6,033,908, both of which are hereby incorporated by reference. It is a primary human retinoblast cell line transduced with an E1 gene segment that complements the production of replication deficient (FG) adenovirus, but is designed to prevent generation of replication competent adenovirus by homologous recombination. Cells of particular interest have been stably transformed with a transgene that encodes the AD5E1A and E1B gene, like PER.C6[®], from 459 bp to 3510 bp inclusive. 293 cells are described in Graham et al., 1977 *J. Gen. Virol* 36:59-72, which is hereby incorporated by reference. As stated above, consideration must be given to the adenoviral sequences present in the complementing cell line used. It is important that the sequences not overlap with that present in the vector if the possibility of recombination is to be minimized.

It has been found that vectors generated in accordance with the above description are more effective in inducing an immune response and, thus, constitute very promising vaccine candidates. More particularly, it has been found that first generation adenoviral vectors in accordance with the above description carrying a codon-optimized HIV gag gene, regulated with a strong heterologous promoter can be used as human anti-HIV vaccines, and are capable of inducing immune responses.

Standard techniques of molecular biology for preparing and purifying DNA constructs enable the preparation of the DNA immunogens of this invention.

A vaccine composition comprising an adenoviral vector in accordance with the instant invention may contain physiologically acceptable components, such as buffer, normal saline or phosphate buffered saline, sucrose, other salts and polysorbate. One preferred formulation has: 2.5-10 mM TRIS buffer, preferably about 5 mM TRIS buffer; 25-100 mM NaCl, preferably about 75 mM NaCl; 2.5-10% sucrose, preferably about 5% sucrose; 0.01 -2 mM MgCl₂; and 0.001%-0.01% polysorbate 80 (plant derived). The pH should range from about 7.0-9.0, preferably about 8.0. One skilled in the art will appreciate that other conventional vaccine excipients may also be used it make the formulation. The preferred formulation contains 5mM TRIS, 75 mM NaCl, 5% sucrose, 1mM MgCl₂, 0.005% polysorbate 80 at pH 8.0 This has a pH and divalent cation composition which is near the optimum for Ad5 stability and minimizes the potential for adsorption of virus to a glass surface. It does not cause tissue irritation upon intramuscular injection. It is preferably frozen until use.

The amount of adenoviral particles in the vaccine composition to be introduced into a vaccine recipient will depend on the strength of the transcriptional and translational promoters used and on the immunogenicity of the expressed gene product. In general, an immunologically or prophylactically effective dose of 1×10^7 to 1×10^{12} particles and preferably about 1×10^{10} to 1×10^{11} particles is administered directly into muscle tissue. Subcutaneous injection, intradermal introduction, impression through the skin, and other modes of administration such as intraperitoneal, intravenous, or inhalation delivery are also contemplated. It is also contemplated that booster vaccinations are to be provided. Following vaccination with HIV adenoviral vector, boosting with a subsequent HIV adenoviral vector and/or plasmid may be desirable. Parenteral administration, such as intravenous, intramuscular, subcutaneous or other means of administration of interleukin-12 protein, concurrently with or subsequent to parenteral introduction of the vaccine compositions of this invention is also advantageous.

The adenoviral vector and/or vaccine plasmids of this invention polynucleotide may be unassociated with any proteins, adjuvants or other agents which impact on the recipients' immune system. In this case, it is desirable for the vector to be in a physiologically acceptable solution, such as, but not limited to, sterile saline or sterile buffered saline. Alternatively, the vector may be associated with an adjuvant known in the art to boost immune responses (i.e., a "biologically effective"

adjuvant), such as a protein or other carrier. Vaccine plasmids of this invention may, for instance, be delivered in saline (e.g., PBS) with or without an adjuvant. Preferred adjuvants are Alum or CRL1005 Block Copolymer. Agents which assist in the cellular uptake of DNA, such as, but not limited to, calcium ions, may also be used to advantage. These agents are generally referred to herein as transfection facilitating reagents and pharmaceutically acceptable carriers. Techniques for coating microprojectiles coated with polynucleotide are known in the art and are also useful in connection with this invention.

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This invention also includes a prime and boost regimen wherein a first adenoviral vector is administered, then a booster dose is given. The booster dose may 10 be repeated at selected time intervals. Alternatively, a preferred inoculation scheme comprises priming with a first adenovirus serotype and then boosting with a second adenovirus serotype. More preferably, the inoculation scheme comprises priming with a first adenovirus serotype and then boosting with a second adenovirus serotype, 15 wherein the first and second adenovirus serotypes are classified within separate subgroups of adenoviruses. The above prime/boost schemes are particularly preferred in those situations where a preexisting immunity is identified to the adenoviral vector of choice. In this type of scheme, the individual or population of individuals is primed with an adenovirus of a serotype other than that to which the preexisting 20 immunity is identified. This enables the first adenovirus to effectuate sufficient expression of the transgene while evading existing immunity to the second adenovirus (the boosting adenovirus) and, further, allows for the subsequent delivery of the transgene via the boosting adenovirus to be more effective. Adenovirus serotype 5 is one example of a virus to which such a scheme might be desirable. In accordance 25 with this invention, therefore, one might decide to prime with a non-group C adenovirus (e.g., Ad12, a group A adenovirus, Ad24, a group D adenovirus, or Ad35, a group B adenovirus) to evade anti-Ad5 immunity and then boost with Ad5, a group C adenovirus. Another preferred embodiment involves administration of a different adenovirus (including non-human adenovirus) vaccine followed by administration of the adenoviral vaccines disclosed. In the alternative, a viral antigen of interest can be first delivered via a viral vaccine other than an adenovirus-based vaccine, and then followed with the adenoviral vaccine disclosed. Alternative viral vaccines include but are not limited to pox virus and venezuelan equine encephilitis virus.

A large body of human and animal data supports the importance of cellular immune responses, especially CTL in controlling (or eliminating) HIV infection. In humans, very high levels of CTL develop following primary infection and correlate

with the control of viremia. Several small groups of individuals have been described who are repeatedly exposed to HIV by remain uninfected; CTL has been noted in several of these cohorts. In the SIV model of HIV infection, CTL similarly develops following primary infection, and it has been demonstrated that addition of anti-CD8 monoclonal antibody abrogated this control of infection and leads to disease progression. This invention uses adenoviral vaccines alone or in combination with plasmid vaccines to induce CTL.

The following non-limiting Examples are presented to better illustrate the invention.

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EXAMPLE 1

Removal of the Intron A Portion of the hCMV Promoter

GMP grade pVIInsHIVgag was used as the starting material to amplify the hCMV promoter. PVIInsHIVgag is a plasmid comprising the CMV immediate-early (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone; see Montgomery et al., supra for a description of the plasmid backbone. The amplification was performed with primers suitably positioned to flank the hCMV promoter. A 5' primer was placed upstream of the Msc1 site of the hCMV promoter and a 3' primer (designed to contain the BgIII recognition sequence) was placed 3' of the hCMV promoter. The resulting PCR product (using high fidelity Taq polymerase) which encompassed the entire hCMV promoter (minus intron A) was cloned into TOPO PCR blunt vector and then removed by double digestion with Msc1 and BgIII. This fragment was then cloned back into the original GMP grade pV1JnsHIVgag plasmid from which the original promoter, intron A, and the gag gene were removed following Msc1 and BgIII digestion. This ligation reaction resulted in the construction of a hCMV promoter (minus intron A) + bGHpA expression cassette within the original pV1JnsHIVgag vector backbone. This vector is designated pVIInsCMV(no intron).

The FLgag gene was excised from pV1JnsHIVgag using BgIII digestion and the 1,526 bp gene was gel purified and cloned into pV1JnsCMV(no intron) at the BgIII site. Colonies were screened using Smal restriction enzymes to identify clones that carried the Flgag gene in the correct orientation. This plasmid, designated pV1JnsCMV(no intron)-FLgag-bGHpA, was fully sequenced to confirm sequence integrity.

Two additional transgenes were also constructed. The plasmid, pV1JnsCMV(no intron)-FLgag-SPA, is identical to pV1JnsCMV(no intron)-FLgag-bGHpA except that the bovine growth hormone polyadenylation signal has been replaced with a short synthetic polyA signal (SPA) of 50 nucleotides in length. The sequence of the SPA is as shown, with the essential components (poly(A) site, (GT)_n, and (T)_n; respectively) underlined:

<u>AATAAA</u>AGATCTTTATTTTCATTAGATCT<u>GTGTG TTGGTTTTTTGTGTG</u> (SEQ ID NO:18).

The plasmid, pV1Jns-mCMV-FLgag-bGHpA, is identical to the pV1JnsCMV(no intron)-FLgag-bGHpA except that the hCMV promoter has been removed and replaced with the murine CMV (mCMV) promoter.

Figure 3 diagrammatically shows the new transgene constructs in comparison with the original transgene.

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EXAMPLE 2

Gag Expression Assay for Modified Gag Transgenes

Gag Elisa was performed on culture supernatants obtained from transient tissue culture transfection experiments in which the two new hCMV-containing plasmid constructs, pV1JnsCMV(no intron)-FLgag-bGHpA and pV1JnsCMV(no intron)-FLgag-SPA, both devoid of intron A, were compared to pV1JnsHIVgag which, as noted above possesses the intron A as part of the hCMV promoter. Table 2 below shows the *in vitro* gag expression data of the new gag plasmids compared with the GMP grade original plasmid. The results displayed in Table 2 show that both of the new hCMV gag plasmid constructs have expression capacities comparable to the original plasmid construct which contains the intron A portion of the hCMV promoter.

Table 2: In vitro DNA transfection of original and new plasmid HTV-1 gag constructs.

Plasmid	μg gag/10e6 COS cells/5μg DNA/48 hr
HIVFL-gagPR9901 ^a	10.8
PVIIns-hCMV-FLgag-bGHpAb	16.6
pV1Jns-hCMV-FLgag-SPA ^{b,c}	12.0

^a GMP grade pV1Jns-hCMVintronA-FLgag-bGHpA.

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EXAMPLE 3

Rodent (Balb/c) Study for Modified gag Transgenes
A rodent study was performed on the two new plasmid constructs
described above – pV1JnsCMV(no intron)-FLgag-bGHpA and pV1JnsCMV(no
intron)-FLgag-SPA - in order to compare them with the construct described above
possessing the intron A portion of the CMV promoter, pV1JnsHIVgag. Gag antibody
and Elispot responses (described in PCT International Application No.
PCT/US00/18332 (WO 01/02607) filed July 3, 2000, claiming priority to U.S.
Provisional Application Serial No. 60/142,631, filed July 6, 1999 and U.S.
Application Serial No. 60/148,981, filed August 13, 1999, all three applications which
are hereby incorporated by reference) were measured. The results displayed in Table
3 below, show that the new plasmid constructs behaved equivalently to the original
construct in Balb/c mice with respect to their antibody and T-cell responses at both
dosages of plasmid DNA tested, 20 μg and 200 μg.

⁵ b New plasmid constructions that have the intron A portion removed from the hCMV promoter.

^c In this construct the bGH terminator has been replaced with the short synthetic polyadenylation signal (SPA)

EXAMPLE 4

Table 3: HIV191: Immunogenicity of V1Jns-gag under different promoter and termination control elements.

DNA	Dose, ug ^b		Anti-p24 Titers (3 Wk PD1) ^c		SFC/10^6 Cells (4 Wk PD1) ^d			
Promoter/terminator		GMT	+SE	-SE	Media	gag197-205	p24	
HIVFL-gagPR9901	200	12800	4652	3412	2(2)	129(19)	30(11)	
(GMP grade)	20	5572	1574	1227	0	56(9)	25(6)	
pV1Jns-hCMV-	200	11143	2831	2257	0	98(5)	12(6)	
FL-gag-bGHpA	20	7352	2808	2032	0	73(9)	11(6)	
pV1Jns-hCMV-	200	16890	5815	4326	1(1)	94(4)	26(7)	
FL-gag-SPA	20	5971	5361	2825	0	85(17)	38(10)	
Naīve	0	123	50	36	0	0	0	

in PBS

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Construction of the Modified Shuttle Vector - "MRKpdelE1 Shuttle"

The modifications to the original Ad5 shuttle vector (pdelE1sp1A; a vector comprising Ad5 sequences from basepairs 1-341 and 3524-5798, with a multiple cloning region between nucleotides 341 and 3524 of Ad5, included the following three manipulations carried out in sequential cloning steps as follows:

- (1) The left ITR region was extended to include the *Pac1* site at the junction between the vector backbone and the adenovirus left ITR sequences. This allow for easier manipulations using the bacterial homologous recombination system.
- 10 (2) The packaging region was extended to include sequences of the wild-type (WT) adenovirus from 342 bp to 450 bp inclusive.
 - (3) The area downstream of pIX was extended 13 nucleotides (i.e., nucleotides 3511-3523 inclusive).

These modifications (Figure 4) effectively reduced the size of the E1 deletion without overlapping with any part of the E1A/E1B gene present in the transformed PER.C6® cell line. All manipulations were performed by modifying the Ad shuttle vector pdelE1sp1A.

Once the modifications were made to the shuttle vector, the changes were incorporated into the original Ad5 adenovector backbones (pAdHVO and pAdHVE3) by bacterial homologous recombination using *E. coli* BJ5183 chemically competent cells.

bi.m. Injections into both quads, 50 µL per quad

cn=10;GMT, geometric mean titer; SE, standard. error

dn=5, pooled spleens; mean of triplicate wells and standard, deviation, in parentheses;

EXAMPLE 5

Construction of Modified Adenovector Backbones (E3+ and E3-)

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The original adenovectors pAdHVO (comprising all Ad5 sequences except those nucleotides encompassing the E1 and E3 regions) and pADHVE3 (comprising all Ad5 sequences except those nucleotides encompassing the E1 region), were each reconstructed so that they contained the modifications to the E1 region. This was accomplished by digesting the newly modified shuttle vector (MRKpdelE1 shuttle) with Pac1 and BstZ1101 and isolating the 2,734 bp fragment which corresponds to the adenovirus sequence. This fragment was co-transformed with DNA from either Cla1 linearized pAdHVO (E3- adenovector) or Cla1 linearized pAdHVE3 (E3+adenovector) into E. coli BJ5183 competent cells. At least two colonies from each transformation were selected and grown in Terrific™ broth for 6-8 hours until turbidity was reached. DNA was extracted from each cell pellet and then transformed into E. coli XL1 competent cells. One colony from each transformation was selected and grown for plasmid DNA purification. The plasmid was analyzed by restriction digestions to identify correct clones. The modified adenovectors were designated MRKpAdHVO (E3- plasmid) and MRKpAdHVE3 (E3+ plasmid). Virus from these new adenovectors (MRKHVO and MRKHVE3, respectively) as well as the old version of the adenovectors were generated in the PER.C6® cell lines to accommodate the following series of viral competition experiments. In addition, the multiple cloning site of the original shuttle vector contained ClaI, BamHI, Xho I, EcoRV, HindIII, Sal I, and Bgl II sites. This MCS was replaced with a new MCS containing Not I, Cla I, EcoRV and Asc I sites. This new MCS has been transferred to the MRKpAdHVO and MRKpAdHVE3 pre-plasmids along with the modification made to the packaging region and pIX gene.

EXAMPLE 6

Analysis of the Effect of the Packaging Signal Extension

To study the effects of the modifications made to the E1 deletion region, the viruses obtained from the original backbone (pAdHVE3) and the new backbone (MRKpAdHVE3) were mixed together in equal MOI ratios (1:1 and 5:5) and passaged through several rounds; see Figure 5, Expt.#1. Both of the viruses in the experiment contained the E3 gene intact and did not contain a transgene. The only difference between the two viruses was within the region of the E1 deletion.

Following the coinfection of the viruses at P1 (passage 1), the mixtures were propagated through an additional 4 passages at which time the cells were harvested.

and the virus extracted and purified by CsCl banding. The viral DNA was extracted and digested with *Hind*III and the digestion products were then radioactively labeled. For the controls, the respective pre-plasmids (pAdHVE3 ("OLD E3+"); MRKpAdHVE3 ("NEW E3+")) were also digested with *Hind*III (and *Pac1* to remove the vector backbone) and subsequently labeled with [³³P]dATP. The radioactively labeled digestion products were subjected to gel electrophoresis and the gel was dried down onto Whatman paper before being exposed to autoradiographic film. Figure 6 clearly shows that the new adenovirus which has the addition made to the packaging signal region has a growth advantage compared with the original adenovirus. In the experiments performed (at either ratio tested), only the digestion bands pertaining to the newly modified virus were present. The diagnostic band of size 3,206 (from the new virus) was clearly present. However, there was no evidence of the diagnostic band of size 2,737 bp expected from the original virus.

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EXAMPLE 7

Analysis of the Effect of the E3 Gene

The second set of the virus competition study involved mixing equal MOI ratio (1:1) of the newly modified viruses, that obtained from MRKpAdHVO and MRKpAdHVE3 (Figure 5, Expt. #2). In this set, both viruses had the new modifications made to the E1 deletion. The first virus (that from MRKpAdHVO) does not contain an E3 gene. The second virus (that from MRKpAdHVE3) does contain the E3 gene. Neither of the viruses contain a transgene. Following coinfection of the viruses, the mixtures were propagated through an additional 4 passages at which time the cells were harvested and the total virus extracted and purified by CsCl banding. The viral DNA was extracted and digested with HindIII and the digestion products were then radioactively labeled. For the controls, the respective pre-plasmids MRKpAdHVO ("NEW E3-"); MRKpAdHVE3 ("NEW E3+") were also digested with HindIII (and Pac1 to remove the vector backbone) and then labeled with [33P]dATP. The radioactively labeled digestion products were subjected to gel electrophoresis and the gel was dried down onto Whatman paper before being exposed to autoradiographic film. Figure 6 shows the results of the viral DNA analysis of the E3+ virus and E3- virus mixing experiment. The diagnostic band corresponding to the E3+ virus (5,665 bp) was present in greater amount compared with the diagnostic band of 3,010 bp corresponding to the E3- virus. This indicates that the virus that contains the E3 gene is able to amplify more rapidly

compared with the virus that does not contain an E3 gene. This increased amplification capacity has been confirmed by growth studies; see Table 4 below.

EXAMPLE 8

Construction of the new shuttle vector containing modified gag transgene – "MRKpdelE1-CMV(no intron)-FLgag-bGHpA"

The modified plasmid pV1JnsCMV(no intron)-FLgag-bGHpA was digested with Msc1 overnight and then digested with Sfi1 for 2 hours at 50°C. The DNA was then treated with Mungbean nuclease for 30 mins at 30°C. The DNA mixture was desalted using the Qiaex II kit and then Klenow treated for 30 mins at 37°C to fully blunt the ends of the transgene fragment. The 2,559 bp transgene fragment was then gel purified. The modified shuttle vector (MRKpdelE1 shuttle) was linearized by digestion with EcoRV, treated with calf intestinal phosphatase and the resulting 6,479 bp fragment was then gel purified. The two purified fragments were then ligated together and several dozen clones were screened to check for insertion of the transgene within the shuttle vector. Diagnostic restriction digestion was performed to identify those clones carrying the transgene in the E1 parallel and E1 anti-parallel orientation. This strategy was followed to clone in the other gag transgenes in the MRKpdelE1 shuttle vector.

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EXAMPLE 9

Construction of the MRK FG Adenovectors

The shuttle vector containing the HIV-1 gag transgene in the E1 parallel orientation, MRKpdelE1-CMV(no intron)-FLgag-bGHpA, was digested with Pac1. 25 The reaction mixture was digested with BsfZ171. The 5,291 bp fragment was purified by gel extraction. The MRKpAdHVE3 plasmid was digested with Cla1 overnight at 37°C and gel purified. About 100 ng of the 5,290 bp shuttle +transgene fragment and ~100 ng of linearized MRKpAdHVE3 DNA were co-transformed into E. coli BJ5183 chemically competent cells. Several clones were selected and grown in 2 ml 30 Terrific™ broth for 6-8 hours, until turbidity was reached. The total DNA from the cell pellet was purified using Qiagen alkaline lysis and phenol chloroform method. The DNA was precipitated with isopropanol and resuspended in 20 µl dH₂0. A 2 µl aliquot of this DNA was transformed into E. coli XL-1 competent cells. A single colony from each separate transformation was selected and grown overnight in 3 ml 35 LB +100 μg/ml ampicillin. The DNA was isolated using Qiagen columns. A positive clone was identified by digestion with the restriction enzyme BstEII which cleaves

within the gag gene as well as the plasmid backbone. The pre-plasmid clone is designated MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA and is 37,498 bp in size. This strategy was followed to generate E3- and E3+ versions of each of the other gag transgene constructions in both E1 parallel and E1 anti-parallel versions. Figures 7A, 7B and 7C show the various combinations of adenovectors constructed.

EXAMPLE 10

Plasmid Competition Studies

A series of plasmid competition studies was carried out. Briefly, the screening of the various combinations of new constructs was performed by mixing equal amounts of each of two competing plasmids. In the experiment shown in Figure 8A, plasmids containing the same transgene but in different orientations were mixed together to create a "competition" between the two plasmids. The aim was to look at the effects of transgene orientation. In the experiment shown in Figure 8B, plasmids containing different polyadenylation signals (but in the same orientation) were mixed together in equal amounts. The aim was to assess effects of polyA signals. Following the initial transfection, the virus was passaged through ten rounds and the viral DNA analyzed by radioactive restriction analysis.

Analysis of the viral species from the plasmid mixing experiment (Figure 8A) showed that adenovectors which had the transgene inserted in the E1 parallel orientation amplified better and were able to out-compete the adenovirus which had the transgene inserted in the E1 anti-parallel orientation. Viral DNA analysis of the mixtures at passage 3 and certainly at passage 6, showed a greater ratio of the virus carrying the transgene in the E1 parallel orientation compared with the E1 anti-parallel version. By passage 10, the only viral species observed was the adenovector with the transgene in the E1 parallel orientation for both transgenes tested (hCMV(no intron)-FLgag-bGHpA and hCMV(no intron)-FLgag-SPA).

Analysis of the viral species from the plasmid mixing experiment #2 (Figure 8B) at passages 3 and 6 showed that the polyadenylation signals tested (bGHpA and SPA) did not have an effect on the growth of the virus. Even at passage 10 the two viral species in the mixture were still present in equal amounts.

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EXAMPLE 11

Virus generation of an enhanced adenoviral construct - "MRK Ad5 HIV-1gag"

The results obtained from the competition study allowed us to make the following conclusions: (1) The packaging signal extension is beneficial; (2) Presence of E3 does enhance viral growth; (3) E1 parallel orientation is recommended; and (4) PolyA signals have no effect on the growth of the adenovirus.

MRK Ad5 HIV-1 gag exhibited the most desirable results. This construct contains the hCMV(no intron)-FLgag-bGHpA transgene inserted into the new E3+ adenovector backbone, MRKpAdHVE3, in the E1 parallel orientation. We have designated this adenovector MRK Ad5 HIV-1 gag. This construct was prepared as outlined below:

The pre-plasmid MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA was digested was Pac1 to release the vector backbone and 3.3 µg was transfected by calcium phosphate method (Amersham Pharmacia Biotech.) in a 6 cm dish containing PER.C6® cells at ~60% confluence. Once CPE was reached (7-10 days), the culture 15 was freeze/thawed three times and the cell debris pelleted. 1 ml of this cell lysate was used to infect into a 6 cm dish containing PER.C6® cells at 80-90% confluence. Once CPE was reached, the culture was freeze/thawed three times and the cell debris pelleted. The cell lysate was then used to infect a 15 cm dish containing PER.C6[®] cells at 80-90% confluence. This infection procedure was continued and expanded at passage 6. The virus was then extracted from the cell pellet by CsCl method. Two bandings were performed (3-gradient CsCl followed by a continuous CsCl gradient). Following the second banding, the virus was dialyzed in A105 buffer. Viral DNA was extracted using pronase treatment followed by phenol chloroform. The viral 25 DNA was then digested with *Hind*III and radioactively labeled with [33P]dATP. Following gel electrophoresis to separate the digestion products the gel was dried down on Whatman paper and then subjected to autoradiography. The digestion products were compared with the digestion products from the pre-plasmid (that had been digested with Pac1/HindIII prior to labeling). The expected sizes were 30 observed, indicating that the virus had been successfully rescued. This strategy was used to rescue virus from each of the various adenovector plasmid constructs prepared.

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EXAMPLE 12 Stability Analyses

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To determine whether the various adenovector constructs (e.g., MRK Ad5 HIV-1 gag) show genetic stability, the viruses were each passaged continually. The viral DNA was analyzed at passages 3, 6 and 10. Each virus maintained its correct genetic structure. In addition, the stability of the MRK Ad5 HIV-1 gag was analyzed under propagation conditions similar to that performed in large scale production. For this analysis, the transfections of MRK Ad5 HIV-1 gag as well as three other adenoviral vectors were repeated and the virus was purified at P3. The three other adenovectors were as follows: (1) that comprising hCMV(no intron)-Flgag with a bGHpA terminator in an E3- adenovector backbone; (2) that comprising hCMV(no intron)-Flgag with a SPA termination signal in an E3+ adenovector backbone, and that comprising a mCMV-Flgag with a bGHpA terminator in an E3+ adenovector backbone. All of the vectors have the transgene inserted in the E1 parallel orientation. Viral DNA was analyzed by radioactive restriction analysis to confirm that it was correct before being delivered to fermentation cell culture for continued passaging in serum-free media. At P5 each of the four viruses were purified and the viral DNA extracted for analysis by the restriction digestion and radiolabeling procedure. This virus has subsequently been used in a series of studies (in vitro gag expression in COS cells, rodent study and rhesus monkey study) as will be described below. The viruses from P5 are shown in Figure 9.

The passaging under serum-free conditions was continued for the MRKHVE3 (transgene-less, obtained from MRKpAdHVE3 pre-plasmid) and the MRKAd5HIV-1gag (obtained from MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA pre-plasmid) viruses. Figure 10 shows viral DNA analysis by radioactive restriction digestion at passage 11 for MRKHVE3, MRKAd5HIV-1gagE3-, and passage 11 and 12 for MRKAd5HIV-1gag. Aside from the first lane which is the DNA marker lane, the next three lanes are virus from the pre-plasmid controls (controls based on the original virus) - MRKpAdHVE3 (also referred to as "pMRKHVE3"), MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA, and pMRKAd5gag(E3-), respectively. As seen in Figure 10, each of the viral DNA samples show the expected bands with no extraneous bands showing. This signifies that there are no major variant adenovirus species present that can be detected by autoradiography.

Figure 11 shows the results of viral competition study between MRKHVE3 and MRKAd5HIV-1gag. These viruses were mixed together at equal MOI (140 viral

particles each; 280 vp total) at passage 6 and continued to be passaged until P11. Aside from the first lane which is the DNA marker lane, the next two lanes are the pre-plasmid controls obtained from MRKpAdHVE3 and MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA. The next two lanes are the viral DNA from the starting viral material at passage six. The last two lanes are the competition studies performed in duplicate. The data in Figure 11 shows the effect the gag transgene in culture. Growth of a MRKAd5gag virus was compared with growth of a "transgene-less" MRKHVE3. These two viruses were infected at the same MOI (i.e. 140 vp each) at passage 6 and then passaged through to passage 11 and the viral pool was analyzed by radioactive restriction analysis. The data shows that one virus did not out compete the other. Therefore, the gag transgene did not show obvious signs of toxicity to the adenovirus.

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Analysis by *Hind*III digestion shows that each virus specie is present in approximately equal amounts. As above, there does not appear to be signs of any extraneous bands. Figure 12 shows higher passage numbers for MRKAd5HIV-1gag grown under serum-containing conditions. The genome integrity again has been maintained and there is no evidence of rearrangements, even at the highest passage level (P21).

Each of the four vectors shown in Figure 9 were analyzed for amplification capacity. Table 4 below shows the QPA analysis used in the estimation of viral amplification ratios at P4. The determination of the amplification ratio for the original HIV-1 gag construct is based on the clinical lot at P12. It has been shown that amplification rates increases with higher passage number for the original virus. The reason for this observation is due to the emergence of variants which exhibit increased growth rates compared to the intact adenovector. With continued passaging of the original Ad gag vector, the level of variants increases and hence amplification rates increase also.

The MRK Ad5 HIV-1 gag virus has also been continually passaged under process conditions (i.e., serum-free media). Viral DNA extracted from passages 11 and 12 show no evidence of rearrangement.

Table 4: Amplification Ratios Based on AEX and QPA Analysis of Virus Amplification from Passage 3 to Passage 4.

Ad gag construct	Amplification Ratio
MRKAd5gag	470
HCMV-Flgag-bGHpA [E3-]	115
HCMV-Flgag-SPA [E3+]	320
mCMV-FLgag-bGHpA [E3+]	420
Original construct *	40 - 50

* This estimation is based on the clinical lot growth characteristics at Passage 12.

EXAMPLE 13

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Analytical Evaluation of the enhanced Ad5 Constructs

To study the effects of the transgene and the E3 gene on virus amplification, the enhanced adenoviral vector, MRK Ad5 HIV-1 gag, along with its transgene-less version (MRKpAdHVE3) and its E3- version (MRK Ad5 HIV-1 gag E3-), was studied for several passages under serum-free conditions. Table 5A shows the amplification ratios determined for passages P3 to P8 for MRK Ad5 HIV-1 gag. Within a certain MOI range, it has been determined that the virus output is directly proportional to the virus input. Therefore, the greater the number of virus particles per cell at infection, the greater the virus amount produced. Viral amplification ratios, on the other hand, are inversely proportional to the virus input. The lower the virus input, the greater the amplification ratio.

Table 5B shows the amplification rates of the new E3+ vector backbone MRKpAdHVE3. It has a significantly lower rate of amplification compared with the gag transgene containing version. This may be contributed to the larger size MRK Ad5 HIV-1 gag since it contains the transgene. This inclusion of the transgene brings the size of the adenovirus closer to the size of a wild type Ad5 virus. It is well known that adenoviruses amplify best when they are at close to their wild type genomic size.

Wild type Ad5 is 35,935 bp. The MRKpAdHVE3 is 32, 905 bp in length. The enhanced adenovector MRK Ad5 HIV-1 gag is 35,453bp (See Figure 14 for vector map; see also Figure 15A-X show the complete pre-adenoviral vector sequence, which includes an additional 2,021 bp of the vector backbone).

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Table 5C shows the amplification rates of the new E3- gag containing virus MRK Ad5 HIV-1 gag E3-. Once again, this virus shows lower growth rate than the enhanced adenoviral vector. This may be attributed to the decreased sized of this virus (due to the E3 gene deletion) compared with wild type Ad5. The MRK Ad5 HIV-1 gag E3- virus is 32,810 bp in length. This can be compared with the wild type Ad5 which is 35,935 bp and MRK Ad5 HIV-1 gag which is 35,453 bp in length.

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Table 5A: Amplification ratios determined by AEX and QPA for MRKAd5gag over several continuous passaging in serum free media. Following P5, two replicate samples were taken (rep-1 and rep-2) and analyzed.

PCT/US01/28861

MRKAd5gag rep1

		ni), Vlability (%)	Harvest Time	Cell Passage	Titer	Ther	QPA	Ratio	Amplification	AEX
	Infection	Harvest	- Իքե-	Number	10 ^{re} vp/ml culture	10° vp/csll	10° TCID _{EO} /ml	AEXCOPA"	Ratio	Internal Control
P4	1.49, 81%	0.58, 50%	44	46	8.7	5.9	1.72	50	470	
	4 00 000								(MOI = 125)	1
P5	1.38, 93%	0.66, 47%	48	49	6.7	4.9	1.38	49	170	
P6	1.04, 94%	0.68, 77%	47	48	5.8	5.6	1.42	41	200	
P7	1.50, 84%	0.96, 61%	49.5	50	3.9	1.4	0.97	40	50	
P7	1.09, 97%	0.76, 59%	50	52	5.2	4.7	1.70	81	170	
P8	1.03, 94%	0.86, 64%	47.5	54	9.0	8.7	1.10	82	310	
P9	0.89, 95%	0.99, 73%	47.5	56	4.4	4,9	1.03	43	175	3.12
P10	1.09, 91%	1.06, 66%	47.5	68	3.0	2.8	1.16	26	100	2.84 2.70
P11	1.19, 88%	0.98, 65%	47	60	3.6	3.0	1.15	31	110	2.60 2.70
P12	0.98, 91%	2 00 000				L				2.70
F12	U.30, 9176	0.85, 63%	47,5	47	5.4	5.5	1.20	45	200	2.85
P13	1.00, 88%	0.70, 67%	49	49	5.8	5.8				2.60
		0.70,0776	""	45	3.0	5.6	1.11	52	210	3,18 3,18
P14	1.94, 92%	0.88, 67%	46	53	8.6	4.4			160	3.28
			L							3.27
P15	0.97, 96%	0.64, 68%	47	47	6.9	7.1	,		250	3,12
		<u> </u>								2.91

Table 5B: Amplification ratios determined by AEX and QPA for MRKHVE3 over several continuous passaging in serum free media. MRKHVE3 is the new vector backbone which does NOT carry a transgene.

MRKHVE3

	Xv (10° cells/r	nl), Vlability (%)	Harvest Time	Cell Passage	Titer	Titer	QPA	Ratio	Amplification	AEX
	Infection	Harvest	hpi	Number	10 ¹⁰ vp/ml culture	10° vp/cett	10° TCID ₅₀ /ml	AEX:QPA	Ratio	Internal Control
P4	1.10, 97%	1.28, 79%	49	54	4.1	3.8	1.70	25	300 (MO) = 125)	
P5	0.92, 89%	1.18,77%	47	48	4.3	4.7	1.24	35	170	
P6	1.55, 86%	1.26, 76%	49.5	50	1.2	0.8	0.56	21	30	
P6	1.09, 97%	1.11,81%	49	52	4.0	3.6	1.16	34	130	
P7	1.17, 91%	1,22,91%	47,5	54	3.7	3,2	0.50	74	110	
P8	0.98, 88%	1.41, 83%	48	56	2.1	2.1	0.47	45	75	3.12
P9	1.20, 89%	1.25, 81%	47,5	58	0.8	0.7	0.29	28	25	2.84 2.70 2.60
P10	0.99, 82%	1.55, 85%	47	60	2.3	2.3	0.43	53	80	2.70 2.70
P11	1.07, 96%	1.25, 83%	48	47	2.7	2.5	0.41	68	90	2.86 2.60
P12	0.80, 91%	1.14, 80%	49.5	49	5.9	7.4	0.48	123	260	3.18 3.18
P13	1.98, 95%	1.14, 85%	45.5	53	5.8	3.0			110	3.28
P14	0.97, 96%	1.03, 98%	48.5	47	9.4	9.7			350	3.27 3.12
P15	0.87, 99%	0.97, 59%	49.5	49	5.3	6.1			218	2.91 2.78 2.52

Table 5C. Amplification ratios determined by AEX and QPA for MRKAd5gag(E3-) over several continuous passaging in serum free media. This construct is identical to the MRKAd5gag construct except that this version is DELETED of the E3 gene.

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MRKAd5qaq(E3-)

	Xv (10° cells/n	nl), Viability (%) Harvest	Harvest Time h.p.l.	Cell Passage Number	Titer 10 ¹⁰ vp/ml culture	Titer 10° vp/cell	QPA 10° TCID _{se} /mi	Ratio AEX:QPA	Amplification Ratio	AEX Internal Control
P4	1.62, 77%	1.12, 62%	47.5	46	2.0	1.2	0.92	20	100 (MOI=125)	illiente Corto
P5	1.16, 92%	0.62, 43%	49	49	3.3	2.9	0.99	34	100	
P6	1.71, 86%	0.20, 10%	49	50	4.7	2.7	1.70	28	100	
P6	1.09, 97%	0.63, 54%	49.5	52	5.4	5.0	1.76	31	180	
P7	1.17, 91%	0.98, 72%	47.50	54	7.1	6.1	0.67	105	220	
P8	0.98, 88%	0.77, 48%	48	56	3.1	3.2	0.66	47	115	3.12 2.84
P9	1.20, 89%	1.03, 72%	48	58	1,8	1.5	0.57	32	55	2.70 2.60
P10	0.99, 82%	0.80, 62%	46.5	60	3.2	3.2	0.68	47	115	2.70 2.70
P11	1.07, 96%	0.98, 70%	48.5	47	5.9	5.5	0.68	87	200	2.86 2.60
P12	0.80, 91%	0.67, 59%	50	49	5.1	6.4	0.72	71	230	3.18 3.18
P13	1.96, 95%	0.91, 59%	45.5	53	7.4	3.8			135	3.28 3.27
P14	0.97, 96%	0.81, 74%	48	47	6.8	7.0			250	3.12 2.91
P15	0.87, 99%	0.84, 56%	49	49	4.8	5.5			196	2.78 2.52

EXAMPLE 14

Gag Expression Analysis of the Novel Constructs

In vitro gag analysis of the MRK Ad5 HIV-1 gag and the original HIV-gag vectors (research and clinical lot) show comparable gag expression. The clinical lot shows only a slightly reduced gag expression level. The most noticeable difference is with the mCMV vector. This vector shows roughly 3 fold lower expression levels compared with the other vectors tested (which all contain hCMV promoters). The mCMV-FLgag with bGHpA assay was performed three times using different propagation and purification lots and it consistently exhibited weaker gag expression.

EXAMPLE 15

Evaluation of MRK Ad5 HIV-1 gag and Other gag-Containing Adenovectors in Balb/c Mice

Cohorts of 10 balb/c mice were vaccinated intramuscularly with escalating doses of MRK Ad5 HIV-1 gag, and the research and clinical lots of original Ad5HIV-1gag. Serum samples were collected 3 weeks post dose 1 and analyzed by anti-p24 sandwich ELISA.

Anti-p24 titers in mice that received MRK Ad5 HIV-1 gag (107 and 109 vp(viral particle) doses) were comparable (Figure 13) to those of the research lot of Ad5HIV-1 gag, for which much of the early rhesus data were generated on. These titers were also comparable when E3 is deleted (MRKAd5hCMVgagbGHpA(E3-)) or SPA is substituted for bGHpA terminator (MRKAd5 hCMV-gag-SPA (E3+)) or murine CMV promoter is used in place of hCMV (MRKAd5 mCMV-gag-bGHpA (E3+)) in the MRKAd5 backbone.

The results shown in Table 7 indicate that the three other vectors (in addition to the preferred vector, MRK Ad5 HIV-1 gag, are also capable of inducing strong anti-gag antibody responses in mice. Interestingly enough, while the mCMV-FLgag construct containing bGHpA and E3+ in an E1 parallel orientation showed lowest gag expression in the COS cell *in vitro* infection (Table 6) in comparison with the other vectors tested, it generated the greatest anti-gag antibody response this *in vivo* Balb/c study. Table 7 also shows a dose response in anti-gag antibody production in both the research and the clinical lot. As expected, the clinical lot shows reduced anti-gag antibody induction at each dosage level compared to the same dosage used for the research lot.

Table 6: In vitro analysis for gag expression in COS cells by Elisa assay.

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Viral Vectors ^a	μg gag/4.8x10e5 COS/10e8 parts/48hr
MRKAd5gag ^b	1.40
Clinical lot Ad5gag ^c	1.28
Research lot Ad5gag ^d	1.32
MCMVFL-gagbGHpA ^e	0.42

^a A_{260nm} absorbance readings taken for viral particle determinations.

^b MRKAd5gag was produced in serum free conditions and purified at P5.

^c Clinical lot# Ad5gagFN0001

²⁵ d Research Ad5FLgag lot# 6399

[°] mCMVFL-gagbGHpA was produced in serum free conditions and purified at P5.

Table 7: mHIV020 Anti-p24 Ab Titers in Balb/c mice (n=10) vaccinated with various Adgag constructs and lots (3 week post dose1).

Group ID	Vaccine	Dose (vp)	GMT	SE upper	SE lower
1 2	^a MRKAd5gag	10^7 10^9	25600 409600	5877 94028	4780 76473
3 4	hCMV FL-gag bGHpA [E3-] →	10^7 10^9	7352 235253	2077 59767	1620 47659
5	hCMV FL-gag SPA [E3+] →	10^7 10^9	12800 310419	9905 99181	236 75165
7 8	^b mCMV FL-gag bGHpA [E3+] →	10^7 10^9	44572 941014	23504 239068	15389 190636
9	^c hCMV FL-gag bGHpA [E3-] ←	10^7 10^9	3676 117627	934 17491	745 15227
11 12 13 14	research lot hCMV intronA FL-gag bGHpA [E3-] <-	10^6 10^7 10^8 10^9	528 14703 58813 204800	262 5274 14942 53232	175 3882 11915 42250
15 16 17 18	clinical lot hCMVintronA FL-gag bGHpA [E3-] <-	10^6 10^7 10^8 10^9	230 4222 19401 89144	82 3405 3939 25187	61 1138 3274 19639
19	Naïve	none	93	7	_6

*2x50 µL i.m. (quad) injections/animal

P.I.s: Youil, Chen, Casimiro

Vaccination: T. Toner, Q. Su

Assav: M. Chen

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^aThe structure of MRKAd5gag is: hCMVFL-gagbGHpA [E3+] ightarrow The <u>same lot</u> of MRKAd5gag used in this rodent study was used in the Rhesus monkey study (Tables 7 and 8).

^bThe same lot of mCMVFL-gagbGHpA[E3+] used in the in vitro study (Table 6) ws used here.

EXAMPLE 16

Comparison of Humoral and Cellular Responses Towards the Original Ad-gag Construct with the New MRK Ad5 HIV-1 gag in Rhesus Monkeys

Cohorts of 3 rhesus monkeys were vaccinated intramuscularly with MRK Ad5 HIV-1 gag or the clinical Ad5gag bulk at two doses, 10^{11} vp and 10^9 vp. Immunizations were conducted at week 0, 4, and 25. Serum and PBMC samples were collected at selected time points. The serum sample were assayed for anti-p24 Ab titers (using competitive based assay) and the PBMCs for antigen-specific IFN-gamma secretion following overnight stimulation with gag 20-mer peptide pool (via ELISpot assay).

The results shown in Table 8 indicate comparable responses with respect to the generation of anti-gag antibodies. The frequencies of gag-specific T cells in

^cThis construct was designed by Volker Sandig. It contains a shorter version of the hCMV promoter than that used in the MRK constructs. The adenovector backbone is identical to the original backbone used in the original Adgag vector. Expression at 10e7 dose from this vector is 7 fold lower then the same dose of the MRKAd5gag and 4 fold lower than the research lot.

peripheral blood assummarized in Table 9 demonstrate a strong cellular immune response generated after a single dose with the new construct MRK Ad5 HIV-1 gag. The responses are also boostable with second dose of the same vector. The vector is also able to induce CD8+ T cell responses (as evident by remaining spot counts after CD4+ depletion of PBMCs) which are responsible for cytotoxic activity.

Table 8 Anti-p24 antibody titers (in mMU/mL) in rhesus macaques immunized with

gag-expressing adenovectors (Protocol HIV203).

Vaccine	Pre	Wk4	Wk8	Wk 12	Wk 16	Wk 20	Wk 25	Wk 28
MRKAd5gag°, 10^11 vp								
97N010	<10	118	5528	11523	7062	21997	ND	51593
97N116	<10	62	772	1447	1562	2174	ND	20029
98X007	<10	66	3353	6156	6845	3719	ND	24031
MR KAd5gag, 10^9 vp								
97N120	<10	51	204	318	366	482	ND	6550
97N144	<10	18	118	274	706	888	ND	7136
98X008	<10	15	444	386	996	1072	ND	12851
Ad5gag ^b , Clinical Lot, 10^11 vp	<u> </u>							
97X001	<10	87	2579	4718	7174	7250	ND	69226
97N146	<10	72	3604	7380	7526	18906	ND	60283
98X009	<10	78	4183	3946	3124	6956	ND	26226
Ad5gag, Clinical Lot, 10^9 vp	 							72
97N020	<10	<10	143	371	390	1821	ND	- 17177
97X003	<10	_<10	39	93	156	596	ND	2053
98X012	<10	81	342	717	956	1558	ND	11861
MRKAd5gag (hCMV, bGHpA, E3+)								
^b orlginal Ac5gag vector (hCMV/intro	n A bGHp	4, E3-), lo#	FN0001					
ND, not determined				1				

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PCT/US01/28861 WO 02/22080

Table 9. Number of gag-specific T cells per million peripheral blood mononuclear cells (PBMCs) in rhesus monkeys immunized with gag-expressing adenovectors. Also included are those frequencies in PBMCs depleted of CD4⁺ T cells.

Grp #	Vaccination	Mankey ID	Ī=4	Wk		Wk		l Wk		5 Wk		5 Wk		3 Wk
·	T=0,4,25 wks		Media	Gog H ^b	Media	Gog H	Media	Goog H	Media	Goog H	Media	Goog H	Media	Gog H
1	MRKAdāgag	97ND10	6 4	89 38	0	395	0	1058 993	0	1174	3	775 76	4	1074 594
	1041 VP	97N010(CD4-) 97N116	l "	396	1 1	609	ŏ	534	4	395	lĭ	261	lŏ	408
		97N116(CD4-)	111	676			ō	593			0	184	0	666
		98X007	10	579	0	1304	3	2193	וו	2118	3	1588	0	2113
		98X007(CD4-)	20	965			0	2675			0	1656	0	1278
2	MRKAdāgag	97N120	5	275	1	249	4	141	4	119	9	206	4	219
	10'9 vp	97N120(CD4-)	11	170	۱.	438	0	85	3		0	75 98	5	219 373
		97N144	3	236 148	6	438	6	318 285	3	256	ND	ND		625
		97N144(CD4-) 98X008	6 4	368	1 1	1090	3	891	4	673	3	473	5	735
		98X008(CD4-)	14	696	\	1030	ŏ	1175	,	0,0	ŏ	391	4	848
3	Adagog dinkad lat	97X001	-	261		485	0	817	-	1220b	1	894	0	1858
	10^11 vo	97X001(CD4-)	10	283	l .'	'	1 3	996	lŤ		Ó	1010	Ŏ	1123
		97N146	3	150	1	465	Ö	339	1	1272	3	1238	3	1785
		97N146(CD4-)	6	133	,	1	0	370			0	654	0	971
		98X009	0	93	3	339	3	559	0	896	1	384	0	1748
		98X009(CD4-)	٥	73	<u> </u>		٥	333			0	225	0	644
4	AdSgag dinical lat	97N020	3	30	1	101	ō	66 15	0	36	0	26 1	0	41 16
	10/9 vp	97ND2D(CD4-) 97XD03	10	29 68	5	134	0	18	١,	38	1 4	38	١٧	81
i		97X003(CD4-)	9	40	1 "	134	Ιŏ	1 6	Ι '	"	l õ	4	ŏ	19
		98X012	5	95	3	54	Ιĭ	34	0	18	١ŏ	20	Ιĭ	121
		98XD12(CD4-)	l ii	70	`		Ò	ii	`		0	8	0	41
5	Naïve	96R041	6	8	1	1	0	D	0	0	0	0 15	1	0
		053F	14	18	5	16.	20	14	19	15	10	15	24	Ľ

Based on either 4x10/5 or 2x10/5 cells per well (depending on spot density)
ND, not determined

Production on peolide control

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Pool of 20-ca populates overlapping by 10 caland encompassing the page sequence

The adenovectors described herein and, particularly, MRK Ad5 HIV-1 gag, represent very promising HIV-gag adenovectors with respect to their enhanced growth characteristics in both serum and, more importantly, in serum-free media conditions. In comparison with the current HIV-1 gag adenovector construct, MRK Ad5 HIV-1 gag shows a 5-10 fold increased amplification rate. We have shown that it is genetically stable at passage 21. This construct is able to generate significant cellular immune responses in vivo even at a relatively low dose of 10^9 vp. The potency of the MRKAd5gag construct is comparable to, if not better than the original HIV-1gag vector as shown in this rhesus monkey study.

EXAMPLE 17 CODON OPTIMIZED HIV-1 POL AND CODON OPTIMZED **HIV-1 POL MODIFICATIONS**

The open reading frames for the various synthetic pol genes disclosed herein comprise coding sequences for the reverse transcriptase (or RT which consists of a polymerase and RNase H activity) and integrase (IN). The protein sequence is based

on that of Hxb2r, a clonal isolate of IIIB; this sequence has been shown to be closest to the consensus clade B sequence with only 16 nonidentical residues out of 848 (Korber, et al., 1998, Human retroviruses and AIDS, Los Alamos National Laboratory, Los Alamos, New Mexico). The skilled artisan will understand after review of this specification that any available HIV-1 or HIV-2 strain provides a potential template for the generation of HIV pol DNA vaccine constructs disclosed herein. It is further noted that the protease gene is excluded from the DNA vaccine constructs of the present invention to insure safety from any residual protease activity in spite of mutational inactivation. The design of the gene sequences for both wildtype (wt-pol) and inactivated pol (IA-pol) incorporates the use of human preferred ("humanized") codons for each amino acid residue in the sequence in order to maximize in vivo mammalian expression (Lathe, 1985, J. Mol. Biol. 183:1-12). As can be discerned by inspecting the codon usage in SEQ ID NOs: 1, 3, 5 and 7, the following codon usage for mammalian optimization is preferred: Met (ATG), Gly (GGC), Lys (AAG), Trp (TGG), Ser (TCC), Arg (AGG), Val (GTG), Pro (CCC), Thr (ACC), Glu (GAG); Leu (CTG), His (CAC), Ile (ATC), Asn (AAC), Cys (TGC), Ala (GCC), Gln (CAG), Phe (TTC) and Tyr (TAC). For an additional discussion relating to mammalian (human) codon optimization, see WO 97/31115 (PCT/US97/02294), which, as noted elsewhere in this specification, is hereby incorporated by reference. It is intended that the skilled artisan may use alternative versions of codon optimization or may omit this step when generating HIV pol vaccine constructs within the scope of the present invention. Therefore, the present invention also relates to non-codon optimized versions of DNA molecules and associated recombinant adenoviral HIV vaccines which encode the various wild type and modified forms of the HIV Pol protein disclosed herein. However, codon optimization of these constructs is a preferred embodiment of this invention.

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A particular embodiment of this portion of the invention comprisies codon optimized nucleotide sequences which encode wt-pol DNA constructs (herein, "wt-pol" or "wt-pol (codon optimized))" wherein DNA sequences encoding the protease (PR) activity are deleted, leaving codon optimized "wild type" sequences which encode RT (reverse transcriptase and RNase H activity) and IN integrase activity. A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:1, the open reading frame being contained from an initiating Met residue at nucleotides 10-12 to a termination codon from nucleotides 2560-2562. SEQ ID NO:1 is as follows:

AGATCTACCA TGGCCCCCAT CTCCCCCATT GAGACTGTGC CTGTGAAGCT GAAGCCTGGC

ATGGATGGCC CCAAGGTGAA GCAGTGGCCC CTGACTGAGG AGAAGATCAA GGCCCTGGTG

	GAAATCTGCA	CTGAGATGGA	GAAGGAGGC	AAAATCTCCA	AGATTGGCCC	CGAGAACCCC
	TACAACACCC	CTGTGTTTGC	CATCAAGAAG	AAGGACTCCA	CCAAGTGGAG	GAAGCTGGTG
	GACTTCAGGG	AGCTGAACAA	GAGGACCCAG	GACTTCTGGG	AGGTGCAGCT	GGGCATCCCC
	CACCCCGCTG	GCCTGAAGAA	GAAGAAGTCT	GTGACTGTGC	TGGATGTGGG	GGATGCCTAC
5	TTCTCTGTGC	CCCTGGATGA	GGACTTCAGG	AAGTACACTG	CCTTCACCAT	CCCCTCCATC
	AACAATGAGA	CCCCTGGCAT	CAGGTACCAG	TACAATGTGC	TGCCCCAGGG	CTGGAAGGGC
	TCCCCTGCCA	TCTTCCAGTC	CTCCATGACC	AAGATCCTGG	AGCCCTTCAG	GAAGCAGAAC
	CCTGACATTG	TGATCTACCA	GTACATGGAT	GACCTGTATG	TGGGCTCTGA	CCTGGAGATT
	GGGCAGCACA	GGACCAAGAT	TGAGGAGCTG	AGGCAGCACC	TGCTGAGGTG	GGGCCTGACC
10	ACCCCTGACA	AGAAGCACCA	GAAGGAGCCC	CCCTTCCTGT	GGATGGGCTA	TGAGCTGCAC
	CCCGACAAGT	GGACTGTGCA	GCCCATTGTG	CTGCCTGAGA	AGGACTCCTG	GACTGTGAAT
	GACATCCAGA	AGCTGGTGGG	CAAGCTGAAC	TGGGCCTCCC	AAATCTACCC	TGGCATCAAG
	GTGAGGCAGC	TGTGCAAGCT	GCTGAGGGGC	ACCAAGGCCC	TGACTGAGGT	GATCCCCCTG
	ACTGAGGAGG	CTGAGCTGGA	GCTGGCTGAG	AACAGGGAGA	TCCTGAAGGA	GCCTGTGCAT
15	GGGGTGTACT	ATGACCCCTC	CAAGGACCTG	ATTGCTGAGA	TCCAGAAGCA	GGGCCAGGGC
	CAGTGGACCT	ACCAAATCTA	CCAGGAGCCC	TTCAAGAACC	TGAAGACTGG	CAAGTATGCC
	AGGATGAGGG	GGGCCCACAC	CAATGATGTG	AAGCAGCTGA	CTGAGGCTGT	GCAGAAGATC
	ACCACTGAGT	CCATTGTGAT	CTGGGGCAAG	ACCCCCAAGT	TCAAGCTGCC	CATCCAGAAG
	GAGACCTGGG	AGACCTGGTG	GACTGAGTAC	TGGCAGGCCA	CCTGGATCCC	TGAGTGGGAG
20	TTTGTGAACA	CCCCCCCCT	GGTGAAGCTG	TGGTACCAGC	TGGAGAAGGA	GCCCATTGTG
	GGGGCTGAGA	CCTTCTATGT	GGATGGGGCT	GCCAACAGGG	AGACCAAGCT	GGGCAAGGCT
	GGCTATGTGA	CCAACAGGGG	CAGGCAGAAG	GTGGTGACCC	TGACTGACAC	CACCAACCAG
	AAGACTGAGC	TCCAGGCCAT	CTACCTGGCC	CTCCAGGACT	CTGGCCTGGA	GGTGAACATT
	GTGACTGACT	CCCAGTATGC	CCTGGGCATC	ATCCAGGCCC	AGCCTGATCA	GTCTGAGTCT
25	GAGCTGGTGA	ACCAGATCAT	TGAGCAGCTG	ATCAAGAAGG	AGAAGGTGTA	CCTGGCCTGG
	GTGCCTGCCC	ACAAGGGCAT	TGGGGGCAAT	GAGCAGGTGG	ACAAGCTGGT	GTCTGCTGGC
	ATCAGGAAGG	TGCTGTTCCT	GGATGGCATT	GACAAGGCCC	AGGATGAGCA	TGAGAAGTAC
	CACTCCAACT	GGAGGGCTAT	GGCCTCTGAC	TTCAACCTGC	CCCCTGTGGT	GGCTAAGGAG
	ATTGTGGCCT	CCTGTGACAA	GTGCCAGCTG	AAGGGGGAGG	CCATGCATGG	GCAGGTGGAC
30	TGCTCCCCTG	GCATCTGGCA	GCTGGACTGC	ACCCACCTGG	AGGGCAAGGT	GATCCTGGTG
	GCTGTGCATG	TGGCCTCCGG	CTACATTGAG	GCTGAGGTGA	TCCCTGCTGA	GACAGGCCAG
	GAGACTGCCT	ACTTCCTGCT	GAAGCTGGCT	GGCAGGTGGC	CTGTGAAGAC	CATCCACACT
	GACAATGGCT	CCAACTTCAC	TGGGGCCACA	GTGAGGGCTG	CCTGCTGGTG	GGCTGGCATC
	AAGCAGGAGT	TTGGCATCCC	CTACAACCCC	CAGTCCCAGG	GGGTGGTGGA	GTCCATGAAC
35	AAGGAGCTGA	AGAAGATCAT	TGGGCAGGTG	AGGGACCAGG	CTGAGCACCT	GAAGACAGCT
	GTGCAGATGG	CTGTGTTCAT	CCACAACTTC	AAGAGGAAGG	GGGGCATCGG	GGGCTACTCC

GCTGGGGAGA GGATTGTGGA CATCATTGCC ACAGACATCC AGACCAAGGA GCTCCAGAAG
CAGATCACCA AGATCCAGAA CTTCAGGGTG TACTACAGGG ACTCCAGGAA CCCCCTGTGG
AAGGGCCCTG CCAAGCTGCT GTGGAAGGGG GAGGGGGCTG TGGTGATCCA GGACAACTCT
GACATCAAGG TGGTGCCCAG GAGGAAGGCC AAGATCATCA GGGACTATGG CAAGCAGATG
GCTGGGGATG ACTGTGTGCC CTCCAGGCAG GATGAGGACT AAAGCCCGGG CAGATCT (SEQ
ID NO:1).

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The open reading frame of the wild type pol construct disclosed as SEQ ID NO:1 contains 850 amino acids, disclosed herein as SEQ ID NO:2, as follows: Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro 10 Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile 15 Pro His Pro Ala Gly Leu Lys Lys Lys Ser Val Thr Val Leu Asp Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln 20 Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Asp Asp Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val 25 Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln 30 Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp 35 Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Asp Gly Ala Ala

Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Glu Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Asp Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys 10 Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Asp Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Asp Asn Gly Ser Asn Phe Thr Gly Ala Thr Val 15 Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Glu Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly 20 Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp 25 Glu Asp (SEO ID NO:2).

The present invention especially relates to an adenoviral vector vaccine which comprises a codon optimized HIV-1 DNA pol construct wherein, in addition to deletion of the portion of the wild type sequence encoding the protease activity, a combination of active site residue mutations are introduced which are deleterious to HIV-1 pol (RT-RH-IN) activity of the expressed protein. Therefore, the present invention preferably relates to an adenoviral HIV-1 DNA pol-based vaccine wherein the construct is devoid of DNA sequences encoding any PR activity, as well as containing a mutation(s) which at least partially, and preferably substantially, abolishes RT, RNase and/or IN activity. One type of HIV-1 pol mutant which is part and parcel of an adenoviral vector vaccine may include but is not limited to a mutated

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DNA molecule comprising at least one nucleotide substitution which results in a point mutation which effectively alters an active site within the RT, RNase and/or IN regions of the expressed protein, resulting in at least substantially decreased enzymatic activity for the RT, RNase H and/or IN functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a HIV-1 DNA pol construct contains a mutation or mutations within the Pol coding region which effectively abolishes RT, RNase H and IN activity. An especially preferable HIV-1 DNA pol construct in a DNA molecule which contains at least one point mutation which alters the active site of the RT, RNase H and IN domains of Pol, such that each activity is at least substantially abolished. Such a HIV-1 Pol mutant will most likely comprise at least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. To this end, an especially preferred HIV-1 DNA pol construct is exemplified herein and contains nine codon substitution mutations which results in an inactivated Pol protein (IA Pol: SEO ID NO:4, Figure 17A-C) which has no PR, RT, RNase or IN activity, wherein three such point mutations reside within each of the RT, RNase and IN catalytic domains. Therefore, an especially preferred exemplification is an adenoviral vaccine which comprises, in an appropriate fashion, a DNA molecule which encodes IA-pol, which contains all nine mutations as shown below in Table 1. An additional preferred amino acid residue for substitution is Asp551, localized within the RNase domain of Pol. Any combination of the mutations disclosed herein may suitable and therefore may be utilized as an IA-Pol-based vaccine of the present invention. While addition and deletion mutations are contemplated and within the scope of the invention, the preferred mutation is a point mutation resulting in a substitution of the wild type amino acid with an alternative amino acid residue.

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	wt aa	aa residue	mutant aa	enzyme function
	Asp	112	Ala	RT
30 35	Asp	187	Ala	RT
	Asp	188	Ala	RT
	Asp .	445	. Ala .	RNase H
	Glu	480	Ala :	RNase H
	Asp	500	Ala	RNase H
	Asp	626	Ala	IN
	Asp	678	Ala	IN
	Glu	714	Ala	IN

It is preferred that point mutations be incorporated into the IApol mutant adenoviral vaccines of the present invention so as to lessen the possibility of altering epitopes in and around the active site(s) of HIV-1 Pol.

To this end, SEQ ID NO:3 discloses the nucleotide sequence which codes for a codon optimized pol in addition to the nine mutations shown in Table 1, disclosed as follows, and referred to herein as "IApol":

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AGATCTACCA TGGCCCCCAT CTCCCCCATT GAGACTGTGC CTGTGAAGCT GAAGCCTGGC ATGGATGGCC CCAAGGTGAA GCAGTGGCCC CTGACTGAGG AGAAGATCAA GGCCCTGGTG GAAATCTGCA CTGAGATGGA GAAGGAGGGC AAAATCTCCA AGATTGGCCC CGAGAACCCC TACAACACCC CTGTGTTTGC CATCAAGAAG AAGGACTCCA CCAAGTGGAG GAAGCTGGTG GACTTCAGGG AGCTGAACAA GAGGACCCAG GACTTCTGGG AGGTGCAGCT GGGCATCCCC CACCCCGCTG GCCTGAAGAA GAAGAAGTCT GTGACTGTGC TGGCTGTGGG GGATGCCTAC TTCTCTGTGC CCCTGGATGA GGACTTCAGG AAGTACACTG CCTTCACCAT CCCCTCCATC AACAATGAGA CCCCTGGCAT CAGGTACCAG TACAATGTGC TGCCCCAGGG CTGGAAGGGC TCCCCTGCCA TCTTCCAGTC CTCCATGACC AAGATCCTGG AGCCCTTCAG GAAGCAGAAC CCTGACATTG TGATCTACCA GTACATGGCT GCCCTGTATG TGGGCTCTGA CCTGGAGATT GGGCAGCACA GGACCAAGAT TGAGGAGCTG AGGCAGCACC TGCTGAGGTG GGGCCTGACC ACCCCTGACA AGAAGCACCA GAAGGAGCCC CCCTTCCTGT GGATGGGCTA TGAGCTGCAC CCCGACAAGT GGACTGTGCA GCCCATTGTG CTGCCTGAGA AGGACTCCTG GACTGTGAAT GACATCCAGA AGCTGGTGGG CAAGCTGAAC TGGGCCTCCC AAATCTACCC TGGCATCAAG GTGAGGCAGC TGTGCAAGCT GCTGAGGGGC ACCAAGGCCC TGACTGAGGT GATCCCCCTG ACTGAGGAGG CTGAGCTGGA GCTGGCTGAG AACAGGGAGA TCCTGAAGGA GCCTGTGCAT GGGGTGTACT ATGACCCCTC CAAGGACCTG ATTGCTGAGA TCCAGAAGCA GGGCCAGGGC CAGTGGACCT ACCAAATCTA CCAGGAGCCC TTCAAGAACC TGAAGACTGG CAAGTATGCC AGGATGAGGG GGGCCCACAC CAATGATGTG AAGCAGCTGA CTGAGGCTGT GCAGAAGATC ACCACTGAGT CCATTGTGAT CTGGGGCAAG ACCCCCAAGT TCAAGCTGCC CATCCAGAAG GAGACCTGGG AGACCTGGTG GACTGAGTAC TGGCAGGCCA CCTGGATCCC TGAGTGGGAG TTTGTGAACA CCCCCCCT GGTGAAGCTG TGGTACCAGC TGGAGAAGGA GCCCATTGTG GGGGCTGAGA CCTTCTATGT GGCTGGGGCT GCCAACAGGG AGACCAAGCT GGGCAAGGCT GGCTATGTGA CCAACAGGGG CAGGCAGAAG GTGGTGACCC TGACTGACAC CACCAACCAG AAGACTGCCC TCCAGGCCAT CTACCTGGCC CTCCAGGACT CTGGCCTGGA GGTGAACATT GTGACTGCCT CCCAGTATGC CCTGGGCATC ATCCAGGCCC AGCCTGATCA GTCTGAGTCT GTGCCTGCCC ACAAGGGCAT TGGGGGCAAT GAGCAGGTGG ACAAGCTGGT GTCTGCTGGC ATCAGGAAGG TGCTGTTCCT GGATGGCATT GACAAGGCCC AGGATGAGCA TGAGAAGTAC CACTCCAACT GGAGGGCTAT GGCCTCTGAC TTCAACCTGC CCCCTGTGGT GGCTAAGGAG

ATTGTGGCCT CCTGTGACAA GTGCCAGCTG AAGGGGAGG CCATGCATGG GCAGGTGGAC
TGCTCCCCTG GCATCTGGCA GCTGGCCTGC ACCCACCTGG AGGGCAAGGT GATCCTGGTG
GCTGTGCATG TGGCCTCCGG CTACATTGAG GCTGAGGTGA TCCCTGCTGA GACAGGCCAG
GAGACTGCCT ACTTCCTGCT GAAGCTGGCT GGCAGGTGGC CTGTGAAGAC CATCCACACT
GCCAATGGCT CCAACTTCAC TGGGGCCACA GTGAGGGCTG CCTGCTGGTG GGCTGGCATC
AAGCAGGAGT TTGGCATCCC CTACAACCCC CAGTCCCAGG GGGTGGTGGC CTCCATGAAC
AAGGAGCTGA AGAAGATCAT TGGGCAGGTG AGGGACCAGG CTGAGCACCT GAAGACAGCT
GTGCAGATGG CTGTGTTCAT CCACAACTTC AAGAGGAAGG GGGGCATCGG GGGCTACTCC
GCTGGGGAGA GGATTGTGGA CATCATTGCC ACAGACATCC AGACCAAGGA GCTCCAGAAG
CAGATCACCA AGATCCAGAA CTTCAGGGTG TACTACAGGG ACTCCAGGAA CCCCCTGTGG
AAGGGCCCTG CCAAGCTGCT GTGGAAGGGG GAGGGGCTT TGGTGATCCA GGACAACTCT
GACATCAAGG TGGTGCCCAG GAGGAAGGCC AAGATCATCA GGGACTATGG CAAGCAGATG
GCTGGGGATG ACTGTGTGGC CTCCAGGCAG GATGAGGACT AAAGCCCGGG CAGATCT (SEQ ID
NO:3).

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15 In order to produce the IA-pol-based adenoviral vaccines of the present invention, inactivation of the enzymatic functions was achieved by replacing a total of nine active site residues from the enzyme subunits with alanine side-chains. As shown in Table 1, all residues that comprise the catalytic triad of the polymerase, namely Asp112, Asp187, and Asp188, were substituted with alanine (Ala) residues 20 (Larder, et al., Nature 1987, 327: 716-717; Larder, et al., 1989, Proc. Natl. Acad. Sci. 1989, 86: 4803-4807). Three additional mutations were introduced at Asp445. Glu480 and Asp500 to abolish RNase H activity (Asp551 was left unchanged in this IA Pol construct), with each residue being substituted for an Ala residue, respectively (Davies, et al., 1991, Science 252:, 88-95; Schatz, et al., 1989, FEBS Lett. 257: 311-25 314; Mizrahi, et al., 1990, Nucl. Acids. Res. 18: pp. 5359-5353). HIV pol integrase function was abolished through three mutations at Asp626, Asp678 and Glu714. Again, each of these residues has been substituted with an Ala residue (Wiskerchen. et al., 1995, J. Virol. 69: 376-386; Leavitt, et al., 1993, J. Biol. Chem. 268: 2113-2119). Amino acid residue Pro3 of SEQ ID NO:4 marks the start of the RT gene. 30 The complete amino acid sequence of IA-Pol is disclosed herein as SEQ ID NO:4 and

The complete amino acid sequence of IA-Pol is disclosed herein as SEQ ID NO:4 and Figure 17A-C, as follows:

Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg

.Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Ser Val Thr Val Leu Ala Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln 10 Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu 15 Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile 20 Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly 25 Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys . Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln 35 Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly

Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:4).

As noted above, it will be understood that any combination of the mutations disclosed above may be suitable and therefore be utilized as an IA-pol-based adenoviral HIV vaccine of the present invention, either when administered alone or in a combined modality regime and/or a prime-boost regimen. For example, it may be possible to mutate only 2 of the 3 residues within the respective reverse transcriptase, RNase-H, and integrase coding regions while still abolishing these enzymatic activities. However, the IA-pol construct described above and disclosed as SEQ ID NO:3, as well as the expressed protein (SEQ ID NO:4;) is preferred. It is also preferred that at least one mutation be present in each of the three catalytic domains.

Another aspect of this portion of the invention are codon optimized HIV-1 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal peptide such as from tPA (tissue-type plasminogen activator) or by a leader peptide such as is found in highly expressed mammalian proteins such as immunoglobulin leader peptides. Any functional leader peptide may be tested for efficacy. However, a preferred embodiment of the present invention, as with HIV-1 Nef constructs shown herein, is to provide for a HIV-1 Pol mutant adenoviral vaccine construction wherein the pol coding region or a portion thereof is operatively linked to a leader peptide, preferably a leader peptide from human tPA. In other words, a codon optimized HIV-1 Pol mutant such as IA-Pol (SEQ ID NO:4) may also comprise a leader peptide at the amino terminal portion of the protein, which may effect cellular trafficking and hence, immunogenicity of the expressed protein within the host cell. As noted in Figure 16A-B, a DNA vector which may be utilized to practice the present invention may be modified by known recombinant DNA methodology to contain a leader signal

peptide of interest, such that downstream cloning of the modified HIV-1 protein of interest results in a nucleotide sequence which encodes a modified HIV-1 tPA/Pol protein. In the alternative, as noted above, insertion of a nucleotide sequence which encodes a leader peptide may be inserted into a DNA vector housing the open reading frame for the Pol protein of interest. Regardless of the cloning strategy, the end result is a polynucleotide vaccine which comprises vector components for effective gene expression in conjunction with nucleotide sequences which encode a modified HIV-1 Pol protein of interest, including but not limited to a HIV-1 Pol protein which contains a leader peptide. The amino acid sequence of the human tPA leader utilized herein is as follows: MDAMKRGLCCVLLLCGAVFVSPSEISS (SEQ ID NO:17). Therefore, another aspect of the present invention is to generate HIV-1 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal peptide such as from tPA. To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity is deleted and a human tPA leader sequence is fused to the 5' end of the coding region. A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame disclosed herein as SEQ ID NO:6.

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To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity is deleted and a human tPA leader sequence is fused to the 5' end of the coding region (herein, "tPA-wt-pol"). A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame being contained from an initiating Met residue at nucleotides 8-10 to a termination codon from nucleotides 2633-2635. SEQ ID NO:5 is as follows:

GATCACCATG GATGCAATGA AGAGAGGCT CTGCTGTGT CTGCTGTGT GTGGAGCAGT
CTTCGTTTCG CCCAGCGAGA TCTCCGCCCC CATCTCCCCC ATTGAGACTG TGCCTGTGAA
GCTGAAGCCT GGCATGGATG GCCCCAAGGT GAAGCAGTGG CCCCTGACTG AGGAGAAGAT
CAAGGCCCTG GTGGAAATCT GCACTGAGAT GGAGAAGGAG GGCAAAAATCT CCAAGATTGG
CCCCGAGAAC CCCTACAACA CCCCTGTGTT TGCCATCAAG AAGAAGGACT CCACCAAGTG
GAGGAAGCTG GTGGACTTCA GGGAGCTGAA CAAGAGGACC CAGGACTTCT GGGAGGTGCA
GCTGGGCATC CCCCACCCCG CTGGCCTGAA GAAGAAGAAG TCTGTGACTG TGCTGGATGT
GGGGGATGCC TACTTCTCTG TGCCCCTGGA TGAGGACTTC AGGAAGTACA CTGCCTTCAC
CATCCCCTCC ATCAACAATG AGACCCCTGG CATCAGGTAC CAGTACAATG TGCTGCCCCA
GGGCTGGAAG GGCTCCCCTG CCATCTTCCA GTCCTCCATG ACCAAGATCC TGGAGCCCTT
CAGGAAGCAG AACCCTGACA TTGTGATCTA CCAGTACATG GATGACCTGT ATGTGGGCTC
TGACCTGGAG ATTGGGCAGC ACAGGACCAA GATTGAGGAG CTGAGGCAGC ACCTGCTGAG

GTGGGGCCTG ACCACCCTG ACAAGAAGCA CCAGAAGGAG CCCCCCTTCC TGTGGATGGG CTATGAGCTG CACCCCGACA AGTGGACTGT GCAGCCCATT GTGCTGCCTG AGAAGGACTC CTGGACTGTG AATGACATCC AGAAGCTGGT GGGCAAGCTG AACTGGGCCT CCCAAATCTA CCCTGGCATC AAGGTGAGGC AGCTGTGCAA GCTGCTGAGG GGCACCAAGG CCCTGACTGA 5 GGTGATCCCC CTGACTGAGG AGGCTGAGCT GGAGCTGGCT GAGAACAGGG AGATCCTGAA GGAGCCTGTG CATGGGGTGT ACTATGACCC CTCCAAGGAC CTGATTGCTG AGATCCAGAA GCAGGGCCAG GGCCAGTGGA CCTACCAAAT CTACCAGGAG CCCTTCAAGA ACCTGAAGAC TGGCAAGTAT GCCAGGATGA GGGGGGCCCA CACCAATGAT GTGAAGCAGC TGACTGAGGC TGTGCAGAAG ATCACCACTG AGTCCATTGT GATCTGGGGC AAGACCCCCA AGTTCAAGCT 10 GCCCATCCAG AAGGAGACCT GGGAGACCTG GTGGACTGAG TACTGGCAGG CCACCTGGAT CCCTGAGTGG GAGTTTGTGA ACACCCCCC CCTGGTGAAG CTGTGGTACC AGCTGGAGAA GGAGCCCATT GTGGGGGCTG AGACCTTCTA TGTGGATGGG GCTGCCAACA GGGAGACCAA GCTGGGCAAG GCTGGCTATG TGACCAACAG GGGCAGGCAG AAGGTGGTGA CCCTGACTGA CACCACCAAC CAGAAGACTG AGCTCCAGGC CATCTACCTG GCCCTCCAGG ACTCTGGCCT 15 GGAGGTGAAC ATTGTGACTG ACTCCCAGTA TGCCCTGGGC ATCATCCAGG CCCAGCCTGA TCAGTCTGAG TCTGAGCTGG TGAACCAGAT CATTGAGCAG CTGATCAAGA AGGAGAAGGT GTACCTGGCC TGGGTGCCTG CCCACAAGGG CATTGGGGGC AATGAGCAGG TGGACAAGCT GGTGTCTGCT GGCATCAGGA AGGTGCTGTT CCTGGATGGC ATTGACAAGG CCCAGGATGA GCATGAGAAG TACCACTCCA ACTGGAGGGC TATGGCCTCT GACTTCAACC TGCCCCCTGT 20 GGTGGCTAAG GAGATTGTGG CCTCCTGTGA CAAGTGCCAG CTGAAGGGGG AGGCCATGCA TGGGCAGGTG GACTGCTCCC CTGGCATCTG GCAGCTGGAC TGCACCCACC TGGAGGGCAA GGTGATCCTG GTGGCTGTGC ATGTGGCCTC CGGCTACATT GAGGCTGAGG TGATCCCTGC TGAGACAGGC CAGGAGACTG CCTACTTCCT GCTGAAGCTG GCTGGCAGGT GGCCTGTGAA GACCATCCAC ACTGACAATG GCTCCAACTT CACTGGGGCC ACAGTGAGGG CTGCCTGCTG 25 GTGGGCTGGC ATCAAGCAGG AGTTTGGCAT CCCCTACAAC CCCCAGTCCC AGGGGGTGGT GGAGTCCATG AACAAGGAGC TGAAGAAGAT CATTGGGCAG GTGAGGGACC AGGCTGAGCA CCTGAAGACA GCTGTGCAGA TGGCTGTGTT CATCCACAAC TTCAAGAGGA AGGGGGGCAT CGGGGGCTAC TCCGCTGGGG AGAGGATTGT GGACATCATT GCCACAGACA TCCAGACCAA GGAGCTCCAG AAGCAGATCA CCAAGATCCA GAACTTCAGG GTGTACTACA GGGACTCCAG 30 GAACCCCCTG TGGAAGGGCC CTGCCAAGCT GCTGTGGAAG GGGGAGGGGG CTGTGGTGAT CCAGGACAAC TCTGACATCA AGGTGGTGCC CAGGAGGAAG GCCAAGATCA TCAGGGACTA TGGCAAGCAG ATGGCTGGGG ATGACTGTGT GGCCTCCAGG CAGGATGAGG ACTAAAGCCC GGGCAGATCT (SEQ ID NO:5).

The open reading frame of the wild type tPA-pol construct disclosed as SEQ ID NO:5 contains 875 amino acids, disclosed herein as SEQ ID NO:6, as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly

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Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Asp Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu 10 Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Asp Asp Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly 15 Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile 20 Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln 25 Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Asp Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly 30 Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr.Glu.Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Asp Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp 35 Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile

Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Asp Cys Thr His Leu Glu 5 Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Asp Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly 10 Val Val Glu Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp 15 Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:6).

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The present invention also relates to a codon optimized HIV-1 Pol mutant contained within a recombinant adenoviral vector such as IA-Pol (SEQ ID NO:4) which comprises a leader peptide at the amino terminal portion of the protein, which may effect cellular trafficking and hence, immunogenicity of the expressed protein within the host cell. Any such adenoviral-based HIV-1 DNA pol mutant disclosed in the above paragraphs is suitable for fusion downstream of a leader peptide, such as a leader peptide including but not limited to the human tPA leader sequence. Therefore, any such leader peptide-based HIV-1 pol mutant construct may include but is not limited to a mutated DNA molecule which effectively alters the catalytic activity of the RT, RNase and/or IN region of the expressed protein, resulting in at least substantially decreased enzymatic activity one or more of the RT, RNase H and/or IN functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a leader peptide/HIV-1 DNA pol construct contains a mutation or mutations within the Pol coding region which effectively abolishes RT, RNase H and IN activity. An especially preferable HIV-1 DNA pol construct is a DNA molecule which contains at least one point mutation which alters the active site and catalytic activity within the RT, RNase H and IN domains of Pol, such that each activity is at least substantially abolished, and preferably totally abolished. Such a HIV-1 Pol mutant will most likely

comprise at least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. An especially preferred embodiment of this portion of the invention relates to a human tPA leader fused to the IA-Pol protein comprising the nine mutations shown in Table 1. The DNA molecule is disclosed herein as SEQ ID NO:7 and the expressed tPA-IA Pol protein comprises a fusion junction as shown in Figure 18. The complete amino acid sequence of the expressed protein is set forth in SEQ ID NO:8. To this end, SEQ ID NO:7 discloses the nucleotide sequence which codes for a human tPA leader fused to the IA Pol protein comprising the nine mutations shown in Table 1 (herein, "tPA-opt-IApol"). The open 10 reading frame begins with the initiating Met (nucleotides 8-10) and terminates with a "TAA" codon at nucleotides 2633-2635. The nucleotide sequence encoding tPA-IAPol is also disclosed as follows: GATCACCATG GATGCAATGA AGAGAGGGCT CTGCTGTGTG CTGCTGCTGT GTGGAGCAGT CTTCGTTTCG CCCAGCGAGA TCTCCGCCC CATCTCCCCC ATTGAGACTG TGCCTGTGAA 15 GCTGAAGCCT GGCATGGATG GCCCCAAGGT GAAGCAGTGG CCCCTGACTG AGGAGAAGAT CAAGGCCCTG GTGGAAATCT GCACTGAGAT GGAGAAGGAG GGCAAAATCT CCAAGATTGG CCCCGAGAAC CCCTACAACA CCCCTGTGTT TGCCATCAAG AAGAAGGACT CCACCAAGTG GAGGAAGCTG GTGGACTTCA GGGAGCTGAA CAAGAGGACC CAGGACTTCT GGGAGGTGCA GCTGGGCATC CCCCACCCG CTGGCCTGAA GAAGAAGAAG TCTGTGACTG TGCTGGCTGT 20 GGGGGATGCC TACTTCTCTG TGCCCCTGGA TGAGGACTTC AGGAAGTACA CTGCCTTCAC CATCCCCTCC ATCAACAATG AGACCCCTGG CATCAGGTAC CAGTACAATG TGCTGCCCCA GGGCTGGAAG GGCTCCCTG CCATCTTCCA GTCCTCCATG ACCAAGATCC TGGAGCCCTT CAGGAAGCAG AACCCTGACA TTGTGATCTA CCAGTACATG GCTGCCCTGT ATGTGGGCTC TGACCTGGAG ATTGGGCAGC ACAGGACCAA GATTGAGGAG CTGAGGCAGC ACCTGCTGAG 25 GTGGGGCCTG ACCACCCCTG ACAAGAAGCA CCAGAAGGAG CCCCCCTTCC TGTGGATGGG CTATGAGCTG CACCCGACA AGTGGACTGT GCAGCCCATT GTGCTGCCTG AGAAGGACTC CTGGACTGTG AATGACATCC AGAAGCTGGT GGGCAAGCTG AACTGGGCCT CCCAAATCTA CCCTGGCATC AAGGTGAGGC AGCTGTGCAA GCTGCTGAGG GGCACCAAGG CCCTGACTGA GGTGATCCCC CTGACTGAGG AGGCTGAGCT GGAGCTGGCT GAGAACAGGG AGATCCTGAA GGAGCCTGTG CATGGGGTGT ACTATGACCC CTCCAAGGAC CTGATTGCTG AGATCCAGAA GCAGGGCCAG GGCCAGTGGA CCTACCAAAT CTACCAGGAG CCCTTCAAGA ACCTGAAGAC TGGCAAGTAT GCCAGGATGA GGGGGGCCCA CACCAATGAT GTGAAGCAGC TGACTGAGGC TGTGCAGAAG ATCACCACTG AGTCCATTGT GATCTGGGGC AAGACCCCCA AGTTCAAGCT GCCCATCCAG AAGGAGACCT GGGAGACCTG GTGGACTGAG TACTGGCAGG CCACCTGGAT 35 CCCTGAGTGG GAGTTTGTGA ACACCCCCC CCTGGTGAAG CTGTGGTACC AGCTGGAGAA GGAGCCCATT GTGGGGGCTG AGACCTTCTA TGTGGCTGGG GCTGCCAACA GGGAGACCAA

GCTGGGCAAG GCTGGCTATG TGACCAACAG GGGCAGGCAG AAGGTGGTGA CCCTGACTGA CACCACCAAC CAGAAGACTG CCCTCCAGGC CATCTACCTG GCCCTCCAGG ACTCTGGCCT GGAGGTGAAC ATTGTGACTG CCTCCCAGTA TGCCCTGGGC ATCATCCAGG CCCAGCCTGA TCAGTCTGAG TCTGAGCTGG TGAACCAGAT CATTGAGCAG CTGATCAAGA AGGAGAAGGT 5 GTACCTGGCC TGGGTGCCTG CCCACAAGGG CATTGGGGGC AATGAGCAGG TGGACAAGCT GGTGTCTGCT GGCATCAGGA AGGTGCTGTT CCTGGATGGC ATTGACAAGG CCCAGGATGA GCATGAGAAG TACCACTCCA ACTGGAGGGC TATGGCCTCT GACTTCAACC TGCCCCCTGT GGTGGCTAAG GAGATTGTGG CCTCCTGTGA CAAGTGCCAG CTGAAGGGGG AGGCCATGCA TGGGCAGGTG GACTGCTCCC CTGGCATCTG GCAGCTGGCC TGCACCCCACC TGGAGGGCAA 10 GGTGATCCTG GTGGCTGTGC ATGTGGCCTC CGGCTACATT GAGGCTGAGG TGATCCCTGC TGAGACAGGC CAGGAGACTG CCTACTTCCT GCTGAAGCTG GCTGGCAGGT GGCCTGTGAA GACCATCCAC ACTGCCAATG GCTCCAACTT CACTGGGGCC ACAGTGAGGG CTGCCTGCTG GTGGGCTGGC ATCAAGCAGG AGTTTGGCAT CCCCTACAAC CCCCAGTCCC AGGGGGTGGT GGCCTCCATG AACAAGGAGC TGAAGAAGAT CATTGGGCAG GTGAGGGACC AGGCTGAGCA 15 CCTGAAGACA GCTGTGCAGA TGGCTGTTT CATCCACAAC TTCAAGAGGA AGGGGGGCAT CGGGGGCTAC TCCGCTGGGG AGAGGATTGT GGACATCATT GCCACAGACA TCCAGACCAA GGAGCTCCAG AAGCAGATCA CCAAGATCCA GAACTTCAGG GTGTACTACA GGGACTCCAG GAACCCCTG TGGAAGGGCC CTGCCAAGCT GCTGTGGAAG GGGGAGGGGG CTGTGGTGAT CCAGGACAAC TCTGACATCA AGGTGGTGCC CAGGAGGAAG GCCAAGATCA TCAGGGACTA 20 TGGCAAGCAG ATGGCTGGGG ATGACTGTGT GGCCTCCAGG CAGGATGAGG ACTAAAGCCC GGGCAGATCT (SEQ ID NO:7).

The open reading frame of the tPA-IA-pol construct disclosed as SEQ ID NO:7 contains 875 amino acids, disclosed herein as tPA-IA-Pol and SEQ ID NO:8, as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Ala Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr

Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu 10 Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr 15 Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala 20 Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile 25 Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe

Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:8).

EXAMPLE 18

CODON OPTIMIZED HIV-1 NEF AND CODON OPTIMIZED HIV-1 NEF MODIFICATIONS

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Codon optimized version of HIV-1 Nef and HIV-1 Nef modifications are essentially as described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International Application PCT/US00/34162, also filed 15 December 15, 2000, both documents which are hereby incorporated by reference. As disclosed within the above-mentioned documents, particular embodiments of codon optimized Nef and Nef modifications relate to a DNA molecule encoding HIV-1 Nef from the HIV-1 jfrl isolate wherein the codons are optimized for expression in a mammalian system such as a human. The DNA molecule which encodes this protein 20 is disclosed herein as SEQ ID NO:9, while the expressed open reading frame is disclosed herein as SEQ ID NO:10. Another embodiment of Nef-based coding regions for use in the adenoviral vectors of the present invention comprise a codon optimized DNA molecule encoding a protein containing the human plasminogen activator (tpa) leader peptide fused with the NH2-terminus of the HIV-1 Nef 25 polypeptide. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:11, while the expressed open reading frame is disclosed herein as SEQ ID NO:12. Another modified Nef optimized coding region relates to a DNA molecule encoding optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and 30 substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175, herein described as opt nef (G2A, LLAA). The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:13, while the expressed open reading frame is disclosed herein as SEQ ID NO:14. An additional embodiment relates to a DNA molecule encoding optimized HTV-1 Nef wherein the amino terminal myristylation 35 site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA), comprises an open reading frame which

encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175, herein referred to as opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, while the expressed open reading frame is disclosed herein as SEQ ID NO:16.

As disclosed in the above-identified documents (U.S. Application Serial No. 09/738,782 and PCT International Application PCT/US00/34162) and reiterated herein, the following nef-based nucleotide and amino acid sequences which comprise the respective open reading frame are as follows:

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1. The nucleotide sequence of the codon optimized version of HIV-1 jrfl nef gene is disclosed herein as SEQ ID NO:9, as shown herein:

GATCTGCCAC CATGGGCGGC AAGTGGTCCA AGAGGTCCGT GCCCGGCTGG TCCACCGTGA
GGGAGAGGAT GAGGAGGGCC GAGCCCGCCG CCGACAGGGT GAGGAGGACC GAGCCCGCCG
CCGTGGGCGT GGGCGCCGTG TCCAGGGACC TGGAGAAGCA CGGCGCCATC ACCTCCTCCA
ACACCGCCGC CACCAACGCC GACTGCGCCT GGCTGGAGGC CCAGGAGGAC GAGGAGGTGG
GCTTCCCCGT GAGGCCCCAG GTGCCCCTGA GGCCCATGAC CTACAAGGGC GCCGTGGACC
TGTCCCACTT CCTGAAGGAG AAGGGCGGCC TGGAGGGCCT GATCCACTCC CAGAAGAGGC
AGGACATCCT GGACCTGTGG GTGTACCACA CCCAGGGCTA CTTCCCCGAC TGGCAGAACT
ACACCCCCGG CCCCGGCATC AGGTTCCCCC TGACCTTCGG CTGGTGCTTC AAGCTGGTGC
CCGTGGAGCC CGAGAAGGTG GAGGAGGCCA ACGAGGGCGA GAACAACTGC CTGCTGCACC
CCATGTCCCA GCACGGCATC GAGGACCCCG AGAAGGAGGT GCTGGAGTGG AGGTTCGACT
CCAAGCTGGC CTTCCACCAC GTGGCCAGGG AGCTGCACCC CGAGTACTAC AAGGACTGCT
AAAGCCCGGG C (SEQ ID NO:9).

Preferred codon usage is as follows: Met (ATG), Gly (GGC), Lys (AAG), Trp (TGG), Ser (TCC), Arg (AGG), Val (GTG), Pro (CCC), Thr (ACC), Glu (GAG); Leu (CTG), His (CAC), Ile (ATC), Asn (AAC), Cys (TGC), Ala (GCC), Gln (CAG), Phe (TTC) and Tyr (TAC). For an additional discussion relating to mammalian (human) codon optimization, see WO 97/31115 (PCT/US97/02294), which is hereby incorporated by reference. See also Figure 19A-B for a comparion of wild type vs. codon optimized nucleotides comprising the open reading frame of HIV-Nef.

The open reading frame for SEQ ID NO:9 above comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662. The open reading frame of SEQ ID NO:9 provides for a 216 amino acid HIV-1 Nef protein expressed through utilization of a codon optimized DNA vaccine vector. The 216 amino acid HIV-1 Nef (jfrl) protein is disclosed herein as SEQ ID NO:10, and as follows:

Met Gly Gly Lys Trp Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val

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HIV-1 Nef is a 216 amino acid cytosolic protein which associates with the inner surface of the host cell plasma membrane through myristylation of Gly-2 (Franchini et al., 1986, Virology 155: 593-599). While not all possible Nef functions have been elucidated, it has become clear that correct trafficking of Nef to the inner plasma membrane promotes viral replication by altering the host intracellular environment to facilitate the early phase of the HIV-1 life cycle and by increasing the infectivity of progeny viral particles. In one aspect of the invention regarding codon-optimized, protein-modified polypeptides, the nef-encoding region of the adenovirus vector of the present invention is modified to contain a nucleotide sequence which encodes a heterologous leader peptide such that the amino terminal region of the expressed protein will contain the leader peptide. The diversity of function that typifies eukaryotic cells depends upon the structural differentiation of their membrane boundaries. To generate and maintain these structures, proteins must be transported from their site of synthesis in the endoplasmic reticulum to predetermined destinations throughout the cell. This requires that the trafficking proteins display sorting signals that are recognized by the molecular machinery responsible for route selection located at the access points to the main trafficking pathways. Sorting decisions for most proteins need to be made only once as they traverse their biosynthetic pathways since their final destination, the cellular location at which they perform their function, becomes their permanent residence. Maintenance of intracellular integrity depends in part on the selective sorting and accurate transport of proteins to their correct destinations. Defined sequence motifs exist in proteins which can act as 'address labels'. A number of sorting signals have

been found associated with the cytoplasmic domains of membrane proteins. An effective induction of CTL responses often required sustained, high level endogenous expression of an antigen. As membrane-association via myristylation is an essential requirement for most of Nef's function, mutants lacking myristylation, by glycine-to-alanine change, change of the dileucine motif and/or by substitution with a tpa leader sequence as described herein, will be functionally defective, and therefore will have improved safety profile compared to wild-type Nef for use as an HIV-1 vaccine component.

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In another embodiment of this portion of the invention, either the DNA vector or the HIV-1 nef nucleotide sequence is modified to include the human tissue-specific plasminogen activator (tPA) leader. As shown in Figure 16A-B, a DNA vector may be modified by known recombinant DNA methodology to contain a leader signal peptide of interest, such that downstream cloning of the modified HIV-1 protein of interest results in a nucleotide sequence which encodes a modified HIV-1 tPA/Nef protein. In the alternative, as noted above, insertion of a nucleotide sequence which encodes a leader peptide may be inserted into a DNA vector housing the open reading frame for the Nef protein of interest. Regardless of the cloning strategy, the end result is a polynucleotide vaccine which comprises vector components for effective gene expression in conjunction with nucleotide sequences which encode a modified HIV-1 Nef protein of interest, including but not limited to a HIV-1 Nef protein which contains a leader peptide. The amino acid sequence of the human tPA leader utilized herein is as follows: MDAMKRGLCCVILLLCGAVFVSPSEISS (SEO ID NO:17).

It has been shown that myristylation of Gly-2 in conjunction with a dileucine motif in the carboxy region of the protein is essential for Nef-induced down regulation of CD4 (Aiken et al., 1994, Cell 76: 853-864) via endocytosis. It has also been shown that Nef expression promotes down regulation of MHCI (Schwartz et al., 1996, Nature Medicine 2(3): 338-342) via endocytosis. The present invention relates in part to DNA vaccines which encode modified Nef proteins altered in trafficking and/or functional properties. The modifications introduced into the adenoviral vector HIV vaccines of the present invention include but are not limited to additions, deletions or substitutions to the nef open reading frame which results in the expression of a modified Nef protein which includes an amino terminal leader peptide, modification or deletion of the amino terminal myristylation site, and modification or deletion of the dileucine motif within the Nef protein and which alter function within the infected host cell. Therefore, a central theme of the DNA molecules and recombinant adenoviral HIV vaccines of the present invention is (1)

host administration and intracellular delivery of a codon optimized nef-based adenoviral HIV vaccine; (2) expression of a modified Nef protein which is immunogenic in terms of eliciting both CTL and Th responses; and, (3) inhibiting or at least altering known early viral functions of Nef which have been shown to promote HIV-1 replication and load within an infected host. Therefore, the nef coding region may be altered, resulting in a DNA vaccine which expresses a modified Nef protein wherein the amino terminal Gly-2 myristylation residue is either deleted or modified to express alternate amino acid residues. Also, the nef coding region may be altered so as to result in a DNA vaccine which expresses a modified Nef protein wherein the dileucine motif is either deleted or modified to express alternate amino acid residues. In addition, the adenoviral vector HIV vaccines of the present invention also relate to an isolated DNA molecule, regardless of codon usage, which expresses a wild type or modified Nef protein as described herein, including but not limited to modified Nef proteins which comprise a deletion or substitution of Gly 2, a deletion or substitution of Leu 174 and Leu 175 and/or inclusion of a leader sequence.

Therefore, specific Nef-based constructs further include the following, as exemplification's and not limitations. For example, the present invention relates to an adenoviral vector vaccine which encodes modified forms of HIV-1, an open reading frame which encodes a Nef protein which comprises a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl) is referred to herein as opt tpanef. The nucleotide sequence comprising the open reading frame of opt tpanef is disclosed herein as SEQ ID NO:11, as shown below:

CATGGATGCA ATGAAGAGAG GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT
TTCGCCCAGC GAGATCTCCT CCAAGAGGTC CGTGCCCGGC TGGTCCACCG TGAGGGGAGAG
GATGAGGAGG GCCGAGCCCG CCGCCGACAG GGTGAGGAGG ACCGAGCCCG CCGCCGTGGG
CGTGGGCGCC GTGTCCAGGG ACCTGGAGAA GCACGGCGCC ATCACCTCCT CCAACACCGC
CGCCACCAAC GCCGACTGCG CCTGGCTGGA GGCCCAGGAG GACGAGGAGG TGGGCTTCCC
CGTGAGGCCC CAGGTGCCCC TGAGGCCCAT GACCTACAAG GGCGCCGTGG ACCTGTCCCA
CTTCCTGAAG GAGAAGGGCG GCCTGGAGGG CCTGATCCAC TCCCAGAAGA GGCAGGACAT
CCTGGACCTG TGGGTGTACC ACACCCAGGG CTACTTCCCC GACTGGCAGA ACTACACCCC
CGGCCCCGGC ATCAGGTTCC CCCTGACCTT CGGCTGGTGC TTCAAGCTGG TGCCCGTGGA
GCCCGAGAAG GTGGAGGAGG CCAACGAGGG CGAGAACAAC TGCCTGCTGC ACCCCATGTC
CCAGCACGGC ATCGAGGACC CCGAGAAGGA GGTGCTGGAG TGGAGGTTCG ACTCCAAGCT
GGCCTTCCAC CACGTGGCCA GGGAGCTGCA CCCCGAGTAC TACAAGGACT GCTAAAGCC
(SEQ ID NO:11).

The open reading frame for SEQ ID NO:11 comprises an initiating methionine

residue at nucleotides 2-4 and a "TAA" stop codon from nucleotides 713-715. The open reading frame of SEQ ID NO:3 provides for a 237 amino acid HIV-1 Nef protein which comprises a tPA leader sequence fused to amino acids 6-216 of HIV-1 Nef, including the dileucine motif at amino acid residues 174 and 175. This 237 amino acid tPA/Nef (jfrl) fusion protein is disclosed herein as SEQ ID NO:12, and is shown as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val 10 Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu 15 Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Leu Leu His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His 20 Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:12). Therefore, this exemplified Nef protein, Opt tPA-Nef, contains both a tPA leader sequence as well as deleting the myristylation site of Gly-2A DNA molecule encoding HIV-1 Nef from the HIV-1 jfrl isolate wherein the codons are optimized for expression in a mammalian system such as a human. 25

In another specific embodiment of the present invention, a DNA molecule is disclosed which encodes optimized HIV-1 Nef wherein the open reading frame of a recombinant adenoviral HIV vaccine encodes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175. This open reading frame is herein described as opt nef (G2A,LLAA) and is disclosed as SEQ ID NO:13, which comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662. The nucleotide sequence of this codon optimized version of HIV-1 jrfl nef gene with the above mentioned modifications is disclosed herein as SEQ ID NO:13,

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GATCTGCCAC CATGGCCGGC AAGTGGTCCA AGAGGTCCGT GCCCGGCTGG TCCACCGTGA GGGAGAGGAT GAGGAGGGCC GAGCCCGCCG CCGACAGGGT GAGGAGGACC GAGCCCGCCG CCGTGGGCCGT GCCGCGCGT TCCAGGGACC TGGAGAAGCA CGGCGCCATC ACCTCCTCCA ACACCGCCGC CACCAACGCC GACTGCGCCT GGCTGGAGGC CCAGGAGGAC GAGGAGGTGG GCTTCCCCGT GAGGCCCCAG GTGCCCCTGA GGCCCATGAC CTACAAGGGC GCCGTGGACC TGTCCCACTT CCTGAAGGAG AAGGGCGGCC TGGAGGGCCT GATCCACTCC CAGAAGAGGC AGGACATCCT GGACCTGTGG GTGTACCACA CCCAGGGGCTA CTTCCCCGAC TGGCAGAACT ACACCCCCGG CCCCGGCATC AGGTTCCCCC TGACCTTCGG CTGGTGCTTC AAGCTGGTGC CCGTGGAGCC CGAGAAGGGC GAGGAGGCCA ACGAGGGCGA GAACAACTGC GCCGCCCACC CCATGTCCCA GCACGGCATC GAGGACCCC AGAAGGAGGT GCTGGAGTGG AGGTTCGACT CCAAGCTGGC CTTCCACCAC GAGGACCCC AGAAGGAGGT GCTGGAGTGG AGGTTCGACT AAAGCCCGGG CTTCCACCAC GTGGCCAGGG AGCTGCACCC CGAGTACTAC AAGGACTGCT AAAGCCCGGG CT (SEQ ID NO:13).

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The open reading frame of SEQ ID NO:13 encodes Nef (G2A,LLAA), disclosed herein as SEQ ID NO:14, as follows:

15 Met Ala Gly Lys Trp Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val 20 Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro 25 Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Ala Ala His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys Ser (SEQ ID NO:14).

An additional embodiment of the present invention relates to another DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA) comprises an open reading frame which encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175 (Ala-195 and Ala-196 in this tPA-based fusion protein). The nucleotide

sequence comprising the open reading frame of opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, as shown below:

CATGGATGCA ATGAAGAGAG GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT
TTCGCCCAGC GAGATCTCCT CCAAGAGGTC CGTGCCCGGC TGGTCCACCG TGAGGGAGAG
GATGAGGAGG GCCGAGCCCG CCGCCGACAG GGTGAGGAGG ACCGAGCCCG CCGCCGTGGG
CGTGGGCGCC GTGTCCAGGG ACCTGGAGAA GCACGGCGCC ATCACCTCCT CCAACACCGC
CGCCACCAAC GCCGACTGCG CCTGGCTGGA GGCCCAGGAG GACGAGGAGG TGGGCTTCCC
CGTGAGGCCC CAGGTGCCCC TGAGGCCCAT GACCTACAAG GGCGCCGTGG ACCTGTCCCA
CTTCCTGAAG GAGAAGGGCG GCCTGGAGGG CCTGATCCAC TCCCAGAAGA GGCAGGACAT
CCTGGACCTG TGGGTGTACC ACACCCAGGG CTACTTCCCC GACTGGCAGA ACTACACCCC
CGGCCCCGGC ATCAGGTTCC CCCTGACCTT CGGCTGGTG TTCAAGCTGG TGCCCGTGGA
GCCCGAGAAG GTGGAGGAGG CCAACGAGGG CGAGAACAAC TGCGCCGCCC ACCCCATGTC
CCAGCACGGC ATCGAGGACC CCGAGAAGGA GGTGCTGGAG TGGAGGTTCG ACTCCAAGCT
GGCCTTCCAC CACGTGGCCA GGGAGCTGCA CCCCGAGTAC TACAAGGACT GCTAAAGCCC
(SEQ ID NO:15).

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The open reading frame of SEQ ID NO:7 encoding tPA-Nef (LLAA), disclosed herein as SEQ ID NO:16, is as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Ala Ala His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:16). An adenoviral vector of the present invention may comprise a DNA sequence, regardless of codon usage, which expresses a wild type or modified Nef protein as described herein, including but not limited to modified Nef proteins which comprise a deletion or substitution of Gly 2, a deletion of substitution of Leu 174 and Leu 175

and/or inclusion of a leader sequence. Therefore, partial or fully codon optimized DNA vaccine expression vector constructs are preferred since such constructs should result in increased host expression. However, it is within the scope of the present invention to utilize "non-codon optimized" versions of the constructs disclosed herein, especially modified versions of HIV Nef which are shown to promote a substantial cellular immune response subsequent to host administration.

Figure 20A-C show nucleotide sequences at junctions between nef coding sequence and plasmid backbone of nef expression vectors V1Jns/nef (Figure 20A), V1Jns/nef(G2A,LLAA) (Figure 20B), V1Jns/tpanef (Figure 20C) and V1Jns/tpanef(LLAA) (Figure 20C, also). 5' and 3' flanking sequences of codon optimized nef or codon optimized nef mutant genes are indicated by bold/italic letters; nef and nef mutant coding sequences are indicated by plain letters. Also indicated (as underlined) are the restriction endonuclease sites involved in construction of respective nef expression vectors. V1Jns/tpanef and V1Jns/tpanef(LLAA) have identical sequences at the junctions.

Figure 21 shows a schematic presentation of nef and nef derivatives. Amino acid residues involved in Nef derivatives are presented. Glycine 2 and Leucine 174 and 175 are the sites involved in myristylation and dileucine motif, respectively.

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EXAMPLE 19

MRKAd5Pol Construction and Virus Rescue

Steps performed in the construction of the vectors, including the pre-adenovirus plasmid in the construction of the vectors, including the pre-adenovirus plasmid denoted MRKAd5pol, is depicted in Figure 22. Briefly, the adenoviral shuttle vector for the full-length inactivated HIV-1 pol gene is as follows. The vector MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) is a derivative of the shuttle vector used in the construction of the MRKAd5gag adenoviral pre-plasmid. The vector contains an expression cassette with the hCMV promoter (no intronA) and the bovine growth hormone polyadenylation signal. The expression unit has been inserted into the shuttle vector such that insertion of the gene of choice at a unique BgIII site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1-/E3+)Cla1 (or MRKpAdHVE3) preplasmid. The vector, similar to the original shuttle vector contains the Pac1 site, extension to the packaging signal region, and extension to the pIX gene. The synthetic full-length codon-optimized HIV-1 pol gene was isolated directly from the plasmid pV1Jns-HIV-pol-inact(opt). Digestion of this plasmid with BgI II releases the pol

gene intact (comprising a codon optimized IA pol sequence as disclosed in SEQ ID NO:3). The pol fragment was gel purified and ligated into the MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) shuttle vector at the BgIII site. The clones were checked for the correct orientation of the gene by using restriction enzymes DraIII/Not1. A positive clone was isolated and named MRKpdel+hCMVmin+FL-pol+bGHpA(s). The genetic structure of this plasmid was verified by PCR, restriction enzyme and DNA sequencing. The pre-adenovirus plasmid was constructed as follows. Shuttle plasmid MRKpdel+hCMVmin+FLpol+bGHpA(S) was digested with restriction enzymes Pac1 and Bst1107 I (or its isoschizomer, BstZ107 I) and then co-transformed into E. coli strain BJ5183 with linearized (Cla1 digested) adenoviral backbone plasmid, MRKpAd(E1-/E3+)Cla1. The resulting pre-plasmid originally named MRKpAd+hCMVmin+FLpol+bGHpA(S)E3+ is now referred to as "pMRKAd5pol". The genetic structure of the resulting pMRKAd5pol was verified by PCR, restriction enzyme and DNA sequence analysis. The vectors were transformed into competent E. coli XL-1 Blue for preparative production. The recovered plasmid was verified by restriction enzyme digestion and DNA sequence analysis, and by expression of the pol transgene in transient transfection cell culture. The complete nucleotide sequence of this pMRKAd5HIV-1pol adenoviral vector is shown in Figure 26 A-AO.

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Generation of research-grade recombinant adenovirus - The pre-adenovirus plasmid, pMRKAd5pol, was rescued as infectious virions in PER.C6® adherent monolayer cell culture. To rescue infectious virus, 12 μ g of pMRKAd5pol was digested with restriction enzyme PacI (New England Biolabs) and 3.3 μ g was transfected per 6 cm dish of PER.C6® cells using the calcium phosphate coprecipitation technique (Cell Phect Transfection Kit, Amersham Pharmacia Biotech Inc.). PacI digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into PER.C6® cells. Infected cells and media were harvested 6 -10 days post-transfection, after complete viral cytopathic effect (CPE) was observed. Infected cells and media were stored at \leq -60°C. This pol containing recombinant adenovirus is referred to herein as "MRKAd5pol". This recombinant adenovirus expresses an inactivated HIV-1 Pol protein as shown in SEQ ID NO:6.

EXAMPLE 20

MRKAd5Nef Construction and Virus Rescue

35 Construction of vector: shuttle plasmid and pre-adenovirus plasmid - Key steps performed in the construction of the vectors, including the pre-adenovirus

plasmid denoted MRKAd5nef, is depicted in Figure 23. Briefly, as shown in Example 19 above, the vector

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MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) is the shuttle vector used in the construction of the MRKAd5gag adenoviral pre-plasmid. It has been modified to contain the *Pac1* site, extension to the packaging signal region, and extension to the pIX gene. It contains an expression cassette with the hCMV promoter (no intronA) and the bovine growth hormone polyadenylation signal. The expression unit has been inserted into the shuttle vector such that insertion of the gene of choice at a unique *Bgl11* site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1-/E3+)Cla1 pre-plasmid. The synthetic full-length codon-optimized HIV-1 nef gene was isolated directly from the plasmid pV1Jns/nef (G2A,LLAA). Digestion of this plasmid with *Bgl11* releases the pol gene intact, which comprises the nucleotide sequence as disclosed in SEQ ID NO:13. The nef fragment was gel purified and ligated into the

MRKpdelE1+CMVmin+BGHpA(str.) shuttle vector at the Bgl11 site. The clones were checked for correction orientation of the gene by using restriction enzyme Scal. A positive clone was isolated and named MRKpdelE1hCMVminFL-nefBGHpA(s). The genetic structure of this plasmid was verified by PCR, restriction enzyme and DNA sequencing. The pre-adenovirus plasmid was constructed as follows. Shuttle plasmid MRKpdelE1hCMVminFL-nefBGHpA(s) was digested with restriction enzymes Pac1 and Bst1107 I (or its isoschizomer, BstZ107 I) and then co-transformed into E. coli strain BJ5183 with linearized (Cla1 digested) adenoviral backbone plasmid, MRKpAd(E1/E3+)Cla1. The resulting pre-plasmid originally named MRKpdelE1hCMVminFL-nefBGHpA(s) is now referred to as "pMRKAd5nef". The genetic structure of the resulting pMRKAd5nef was verified by PCR, restriction enzyme and DNA sequence analysis. The vectors were transformed into competent E. coli XL-1 Blue for preparative production. The recovered plasmid was verified by restriction enzyme digestion and DNA sequence analysis, and by expression of the nef transgene in transient transfection cell culture. The complete nucleotide sequence of this pMRKAd5HIV-1nef adenoviral vector is shown in Figure 27A-AM.

Generation of research-grade recombinant adenovirus - The pre-adenovirus plasmid, pMRKAd5nef, was rescued as infectious virions in PER.C6[®] adherent monolayer cell culture. To rescue infectious virus, 12 μg of pMRKAdnef was digested with restriction enzyme Pac1 (New England Biolabs) and 3.3 μg was transfected per 6 cm dish of PER.C6[®] cells using the calcium phosphate coprecipitation technique (Cell Phect Transfection Kit, Amersham Pharmacia Biotech

Inc.). Pac1 digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into PER.C6®cells. Infected cells and media were harvested 6-10 days post-transfection, after complete viral cytopathic effect (CPE) was observed. Infected cells and media were stored at \leq -60°C. This nef containing recombinant adenovirus is now referred to as "MRKAd5nef".

EXAMPLE 21

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Construction of Murine CMV Promoter Containing Shuttle Vectors for Inactivated Pol and Nef/G2A,LLAA

The murine CMV (mCMV) was amplified from the plasmid pMH4 (supplied by Frank Graham, McMaster University) using the primer set: mCMV (Not I) Forward: 5'-ATA AGA ATG CGG CCG CCA TAT ACT GAG TCA TTA GG-3' (SEQ ID NO: 20); mCMV (Bgl II)Reverse: 5'-AAG GAA GAT CTA CCG ACG CTG GTC GCG CCT C-3' (SEQ ID NO:21). The underlined nucleotides represent the Not I and the Bgl II sites respectively for each primer. This PCR amplicon was used for the construction of the mCMV shuttle vector containing the transgene in the E1 parallel orientation. The hCMV promoter was removed from the original shuttle vector (containing the hCMV-gag-bGHpA transgene in the E1 parallel orientation) by digestion with Not I and Bgl II. The mCMV promoter (Not I/Bgl II digested PCR product) was inserted into the shuttle vector in a directional manner. The shuttle vector was then digested with Bgl II and the gag reporter gene (Bgl II fragment) was re-inserted back into the shuttle vector. Several clones were screened for correct orientation of the reporter gene. For the construction of the mCMV-gag in the E1 antiparallel orientation, the mCMV promoter was amplified from the plasmid pMH4 using the following primer set: mCMV (Asc I) Forward: 5'- ATA AGA ATG GCG CGC CAT ATA CTG AGT CAT TAG G (SEQ ID NO:22); mCMV (Bgl II) Reverse: 5' AAG GAA GAT CTA CCG ACG CTG GTC GCG CCT C (SEQ ID NO:23). The underlined nucleotides represent the Asc I and Bgl II sites, respectively for each primer. The shuttle vector containing the hCMV-gag transgene in the E1 antiparallel orientation was digested with Asc1 and Bgl11 to remove the hCMV-gag portion of the transgene. The mCMV promoter (Asc1/Bgl11 digested PCR product) was inserted into the shuttle vector in a directional manner. The vector was then digested with Bgl11 and the gag reporter gene (Bgl11 fragment) was re-inserted. Several clones were screened for correct orientation of the reporter gene. For each of the full length IA pol and full length nef/G2A,LLAA genes, cloning was performed using the unique

 $Bgl ext{ II}$ site within the mCMV-bGHpA shuttle vector. The pol and nef genes were excised from their respective pV1Jns plasmids by $Bgl ext{ II}$ digestion.

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EXAMPLE 22

Construction of mCMV Full Length Inactivated Pol and Full Length nef/G2A.LLAA Adenovectors

Each of these transgenes of Example 21 were inserted into the modified shuttle vector in both the E1 parallel and E1 anti-parallel orientations. *Pac1* and *BstZ110I* digestion of each shuttle vector was performed and each specific transgene fragment containing the flanking Ad5 sequences was isolated and co-transformed with *Cla* I digested MRKpAd5(E3+) or MRKpAd5(E3-) adenovector plasmids via bacterial homologous recombination in BJ5183 *E. coli* cells. Recombinant preplasmid adenovectors containing the various transgenes in both the E3- and E3+ versions (and in the E1 parallel and E1 antiparallel orientations) were subsequently prepared in large scale following transformation into XL-1 Blue *E. coli* cells and analyzed by restriction analysis and sequencing.

EXAMPLE 23

Construction of hCMV-tpa-nef (LLAA) Adenovector

The tpa-nef gene was amplified out from GMP grade pV1Ins-tpanef (LLAA) vector using the primer sets: Tpanef (BamHI) F 5'-ATT GGA TCC ATG GAT GCA ATG AAG AGA GGG (SEQ ID 24); Tpanef (BamHI) R 5'-ATA GGA TCC TTA GCA GTC CTT GTA GTA CTC G (SEQ ID NO:25). The resulting PCR product was digested with BamHI, gel purified and cloned into the Bgl II site of MRKAd5CMV-bGHpA shuttle vector (Bgl II digested and calf intestinal phosphatase treated). Clones containing the tpanef (LLAA) gene (see SEQ ID NO:15 for complet coding region) in the correct orientation with respect to the hCMV promoter were selected following Sca I digestion. The resulting MRKAd5tpanef shuttle vector was digested with Pac I and Bst Z1101 and cloned into the E3+ MRKAd5 adenovector via bacterial

EXAMPLE 24

Immunogenicity of MRKAd5pol and MRKAd5nef Vaccine

Materials and Methods - Rodent Immunization - Groups of N=10 BALB/c

mice were immunized i.m. with the following vectors: (1) MRKAd5hCMV-IApol

(E3+) at either 10^7 vp and 10^9 vp; and (2) MRKAd5hCMV-IApol (E3-) at either

homologous recombination techniques.

10^7 vp and 10^9 vp. At 7 weeks post dose, 5 of the 10 mice per cohort were boosted with the same vector and dose they initially received. At 3 weeks post the second does, sera and spleens were collected from all the animals for RT ELISA and IFNg ELIspot analyses, respectively. For all rodent immunizations, the Ad5 vectors were diluted in 5 mM Tris, 5% sucrose, 75 mM NaCl, 1 mM MgCl2, 0.005% polysorbate 80, pH 8.0. The total dose was injected to both quadricep muscles in 50 μL aliquots using a 0.3-mL insulin syringe with 28-1/2G needles (Becton-Dickinson, Franklin Lakes, NJ).

Groups of N=10 C57/BL6 mice were immunized i.m. with the following vectors: (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+) at either 10^7 vp and 10^9 vp; (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10^7 vp and 10^9 vp; and (3) MRKAd5mCMV-tpanef(LLAA) (E3+) at either 10^7 vp and 10^9 vp. At 7 weeks post dose, 5 of the 10 mice per cohort were boosted with the same vector and dose they initially received. At 3 weeks post the second does, sera and spleens were collected from all the animals for RT ELISA and IFNg ELIspot analyses, respectively.

Non-human Primate immunization - Cohorts of 3 rhesus macaques (2-3 kg) were vaccinated with the following Ad vectors: (1) MRKAd5hCMV-IApol (E3+) at either 10^9 vp and 10^11 vp dose; and (2) MRKAd5hCMV-IApol (E3-) at either 10^9 vp and 10^11 vp; (3) MRKAd5hCMV-nef(G2A,LLAA) (E3+) at either 10^9 vp and 10^11 vp; and (4) MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10^9 vp and 10^11 vp. The vaccine was administered to chemically restrained monkeys (10 mg/kg ketamine) by needle injection of two 0.5 mL aliquots of the Ad vectors (in 5 mM Tris, 5% sucrose, 75 mM NaCl, 1 mM MgCl₂, 0.005% polysorbate 80, pH 8.0) into both deltoid muscles. The animals were immunized twice at a 4 week interval (T=0, 4 weeks).

Murine anti-RT and anti-nef ELISA - Anti-RT titers were obtained following standard secondary antibody-based ELISA. Maxisorp plates (NUNC, Rochester; NY) were coated by overnight incubation with 100 μL of 1 μg/mL HIV-1 RT protein (Advanced Biotechnologies, Columbia, MD) in PBS. For anti-nef ELISA, 100 uL of 1 ug/mL HIV-1 nef (Advanced Biotechnologies, Columbia, MD) was used to coat the plates. The plates were washed with PBS/0.05% Tween 20 using Titertek MAP instrument (Hunstville, AL) and incubated for 2 h with 200 μL/well of blocking solution (PBS/0.05% tween/1% BSA). An initial serum dilution of 100-fold was performed followed by 4-fold serial dilution. 100-μL aliquots of serially diluted samples were added per well and incubated for 2 h at room temperature. The plates

were washed and 100 μ L of 1/1000-diluted HRP-rabbit anti-mouse IgG (ZYMED, San Francisco, CA) were added with 1 h incubation. The plates were washed thoroughly and soaked with 100 μ L 1,2-phenylenediamine dihydrochloride/hydrogen peroxide (DAKO, Norway) solution for 15 min. The reaction was quenched by adding 100 μ L of 0.5M H₂SO4 per well. OD₄₉₂ readings were recorded using Titertek Multiskan MCC/340 with S20 stacker. Endpoint titers were defined as the highest serum dilution that resulted in an absorbance value of greater than or equal to 0.1 OD₄₉₂ (2.5 times the background value).

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Non-human primate and murine ELIspot assays - The enzyme-linked immuno-spot (ELISpot) assay was utilized to enumerate antigen-specific INFy-10 secreting cells from mouse spleens (Miyahira, et al. 1995, J. Immunol, Methods 181:45-54) or macaque PBMCs. Mouse spleens were pooled from 5 mice/cohort and single cell suspensions were prepared at 5x10⁶/mL in complete RPMI media (RPMI1640, 10% FBS, 2mM L-glutamine, 100U/mL Penicillin, 100 u/mL 15 streptomycin, 10 mM Hepes, 50 uM β-ME). Rhesus PBMCs were prepared from 8-15 mL of heparinized blood following standard Ficoll gradient separation (Coligan, et al, 1998, Current Protocols in Immunology. John Wiley & Sons, Inc.). Multiscreen opaque plates (Millipore, France) were coated with 100 µL/well of either 5 µg/mL purified rat anti-mouse IFN-y IgG1, clone R4-6A2 (Pharmingen, San Diego, CA), or 20 15 ug/mL mouse anti-human IFN-γ IgG_{2a} (Cat. No. 1598-00, R&D Systems, Minneapolis, MN) in PBS at 4°C overnight for murine or monkey assays, respectively. The plates were washed with PBS/penicillin/streptomycin and blocked with 200 µL/well of complete RPMI media for 37 °C for at least 2 h.

To each well, 50 μL of cell samples (4-5x10⁵ cells per well) and 50 μL of the antigen solution were added. To the control well, 50 μL of the media containing DMSO were added; for specific responses, either selected peptides or peptide pools (4 ug/mL per peptide final concentration) were added. For BALB/c mice immunized with the pol constructs, stimulation was conducted using a pool of CD4⁺-epitope containing 20-mer peptides (aa21-40, aa411-430, aa641-660, aa731-750, aa771-790) or a pool of CD8⁺-epitope containing peptides (aa201-220, aa311-330, aa781-800). For C57/BL6 mice immunized with the nef construct, either aa51-70 (CD8⁺ T cell epitope) or aa81-100 (CD4⁺) peptide derived from the nef sequence was added for specific stimulation. In monkeys, the responses against pol were evaluated using two pools (L and R) of 20-aa peptides that encompass the entire pol sequence and overlap by 10 amino acids. In monkeys vaccinated with the nef constructs, a single pool containing 20-mer peptides covering the entire HIV-1 nef sequence and overlapping

by 10 aa was used. Each sample/antigen mixture was performed in triplicate wells for murine samples or in duplicate wells for rhesus PBMCs. Plates were incubated at 37°C, 5% CO₂, 90% humidity for 20-24 h. The plates were washed with PBS/0.05% Tween 20 and incubated with 100 μL/well of either 1.25 μg/mL biotin-conjugated rat anti-mouse IFN-γ mAb, clone XMG1.2 (Pharmingen) or of 0.1 ug/mL biotinylated anti-human IFN-gamma goat polyclonal antibody (R&D Systems) at 4°C overnight. The plates were washed and incubated with 100 μL/well 1/2500 dilution of strepavidin-alkaline phosphatase conjugate (Pharmingen) in PBS/0.005% Tween/5% FBS for 30 min at 37 °C. Spots were developed by incubating with 100 μL/well 1-step NBT/BCIP (Pierce Chemicals) for 6-10 min. The plates were washed with water and allowed to air dry. The number of spots in each well was determined using a dissecting microscope and the data normalized to 10⁶ cell input.

Non-human Primate anti-RT ELISA - The pol-specific antibodies in the monkeys were measured in a competitive RT EIA assay, wherein sample activity is determined by the ability to block RT antigen from binding to coating antibody on the plate well. Briefly, Maxisorp plates were coated with saturating amounts of pol positive human serum (#97111234). 250 uL of each sample is incubated with 15 uL of 266 ng/mL RT recombinant protein (in RCM 563, 1% BSA, 0.1% tween, 0.1% NaN₃) and 20 uL of lysis buffer (Coulter p24 antigen assay kit) for 15 min at room temperature. Similar mixtures are prepared using serially diluted samples of a standard and a negative control which defines maximum RT binding. 200 uL/well of each sample and standard were added to the washed plate and the plate incubated 16-24 h at room temperature. Bound RT is quantified following the procedures described in Coulter p24 assay kit and reported in milliMerck units per mL arbitrarily defined by the chosen standard.

Results - Rodent Studies - BALB/c mice (n=5 mice/cohort) were immunized once or twice with varying doses of MRKAd5hCMV-IApol(E3+) and MRKAd5hCMV-IApol(E3-). At 3 weeks after the second dose, Anti-pol IgG levels were determined by an ELISA assay using RT as a surrogate antigen. Cellular response were quantified via IFNy ELISpot assay against pools of pol-epitope containing peptides. The results of these assays are summarized in Table 10. The results indicate that the mouse vaccinees exhibited detectable anti-RT IgGs with an adenovector dose as low as 10^7 vp. The humoral responses are highly dose-dependent and are boostable with a second immunization. One or two doses of either pol vectors elicit high frequencies of antigen-specific CD4⁺ and CD8⁺ T cells; the responses are weakly dose-dependent but are boostable with a second immunization.

Table 10. Immunogenicity of MRKAd5pol Vectors in BALB/c mice.

				Ап	ti-RT IgG Tite	ers"	S	FC/10^6 cel	s°
Group	Vaccine	Dose	No. of Doses	GMT	+SE	-SE	Medlum	CD4+ peptide pool	CD8+ peptide pool
1	MRKAdShCMVFLpol (E3+)	10^7 vp	2 1	310419 919	301785 372	153020 265	1(1) 1(1)	75(4) 72(9)	2313(67) 533(41)
2	MRKAd5hCMVFLpol (E3+)	10^9 vp	2	1638400 ^b 713155	0 528520	0 303555	2(2) 1(1)	114(9) 48(7)	2063(182) 733(89)
3	MRKAd5hCMVFLpol (E3-)	10^7 vp	2	310419 6400	386218 14013	172097 4393	0(0) 10(8)	223(7) 141(21)	2607(27) 409(28)
4	MRKAd5hCMVFLpol (E3-)	10 ^9 vp	2	1638400 ^b 1241675 ^b	0 396725	0 300661	1(1) 0(0)	160(13) 39(13)	2385(11) 833(83)
5	Naïve	none	none	57	9	7	9(2)	11(4)	10(1)

*GMT, geometric mean titler of the cohort of 5 mice; SE, standard error of the gemetric mean

5 C57/BL6 mice were immunized once or twice with varying doses of MRKAd5hCMV-nef(G2A,LLAA) (E3+), MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10^7 vp and(3) MRKAd5mCMV-tpanef(LLAA) (E3+) at either 10^7 vp and 10^9 vp. The immune response were analyzed using similar protocols and the results are listed in Table 11. While anti-nef IgG responses could not be detected in this model system with any of the constructs, there are strong indications of a cellular immunity generated against nef using the ELIspot assay.

Table 11. Immunogenicity of MRKAd5nef Vectors in C57/BL6 mice.

				An	ti-nef IgG Tite	ers"	9	FC/10^6 cell	s ^b
Group	Vaccine	Dose	No. of Doses	GMT	+SE	-SE	Medium	aa51-70 CD8+	aa81-100 CD4+
1	MRKAd5hCMVFLnef (E3+)	10^7 vp	2	174	70	50	1(1)	23(1)	1(1)
			1	132	42	32	0(0)	0(0)	0(0)
2.	MRKAd5hCMVFLnef (E3+)	10^9 vp	2	174	70	50	0(0)	61(7)	4(2)
			1	132	42	32	1(1)	62(7)	3(1)
3	MRKAd5mCMVFLnef (E3+)	10^7 vp	2	132	42	32	3(1)	15(5)	5(2)
			1	115	46	33	3(2)	3(2)	4(2)
4	MRKAd5mCMVFLnet (E3+)	10'9 vp	2	132	42	32	4(2)	83(13)	5(1)
			1	132	42	32	2(1)	29(2)	4(0)
5	MRKAd5mCMVtpanef(E3+)	10^7 vp	2	132	42	32	3(2)	14(2)	5(1)
			1	100	0	0	3(1)	13(4)	10(3)
6	MRKAd5mCMVtpanef(E3+)	10^9 vp	2	230	170	98	3(2)	145(29)	4(0)
		1	1	115	46	33	7(1)	151(14)	10(0)
7	Naïve	none	none	152	78	52 ·	· 21(2)	· 18(6)	26(3)

*GMT, geometric mean titer of the cohort of 5 mice; SE, standard error of the gemetric mean

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Monkey Studies - Cohorts of 3 rhesus macaques were immunized with 2 doses of MRKAd5hCMV-IApol(E3+) and MRKAd5hCMV-IApol(E3-). The number of antigen-specific T cells (per million PBMCs) were enumerated using one of two

^bNear or at the upper limit of the serial dilution; hence, could be greater than this value

No. of Spot-forming Cells per million spleonoytes; mean values of triplicates are reported along with standard errors in parenthesis.

No. of spot-forming cells per million spleonoytes; mean values of triplicates are reported along with standard errors in parenthesis.

peptide pools (L and R) that cover the entire pol sequence; the results are listed in Table 12. Moderate-to-strong T cell responses were detected in the vaccinees using either constructs even at a low dose of 10^9 vp. Longitudinal analyses of the anti-RT antibody titers in the animals suggest that the pol transgene product is expressed efficiently to elicit a humoral response (Table 13). It would appear that generally higher immune responses were observed in animals that received the E3- construct compared to the E3+ virus.

Table 12. Pol-specific T Cell Responses in MRKAd5pol Immunized Rhesus

10 Macaques.

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Vaccine (T=0,4 wks)	Monk #		Prebleed	1		T=4			T=7			T=16	
		Mock	Pol L	Pol R	Mock	Pol L	Pol R	Mock	Pal L	Pol R	Mock	Pol L	Pol R
MRKAd5hCMV-lAcct(E3+)	99C100	1	0	0	1	38	31	0	52	146	0	49	715
10411 vp	99C215	1 1	2	2	10	98	249	1	109	305	22	88	250
.5	99D201	5	5	4	6	149	95	0	40	35	0	35	18
MRKAd5hCMV-IApol(E3+)	99D212	0	2	0	4	331	114	0	58	14	0	6	6
10/9 Vp	99D180	0	4	2	0	19	192	4	36	156	5	38	106
	99C201	8	5	21	6	62	62	0	18	32	١١	14	65
MRKAc5hCMV+Apd(E3-)	99D239	5	2	2	20	82	172	1	66	114	9	21	40
10/11 VD	99C186	4	12	6	5	120	421	2	271	489	16	875	530
	99C084	1	8	9	8	84	464	0	14	236	1	24	264
MRKAd5hCMV-IAcol(E3-)	CC7C	10	10	8	12	724	745	4	322	376	4	188	176
10/9 vp	ထားမ	2	0	1 1	5	474	468	0	232	212	0	101	121
	CD11	6	6	12	10	98	110	5	60	80	8	25	34
Nave	0830	nd	nd	nd	nd	nd	nd	4	2	2_	2	1	2

nd, not determined Reported are SFC per million PBMCs; mean of duplicate wells

Table 13. Anti-RT Ig Levels in MRKAd5pol Immunized macaques.

RT ANTIBODY ASSAY TITERS IN mMU/	mL			
Vaccine/Monkey Tag	T =4	T =7	T=12	T=16
MRKAd5hCMV-IApol(E3+), 10^11 vp				
99C100	61	1999	5928	4768
99C215	81	1541	2356	2767
99D201	53	336	539	387
MRKAd5hCMV-IApol(E3+), 10^9 vp				
99D212	10	40	49	68
99D180	<10	36	79	93
99C201	<10	37	71	76
MRKAd5hCMV-IApol(E3-), 10^11 vp				
99D239	44	460	1234	1015
99C186	21	· 233 ·	480	345
990084	235	2637	2858	1626
MRK Ad5hCMV-IApol(E3-), 10^9 vp	 			
CC7C	32	175	306	235
@16	20	140	273	419
Φ11	15	112	149	237
	<u>. </u>			

When rhesus macaques were immunized i.m. with two doses of MRKAd5nef

5 constructs, vigorous T cell responses ranging from 100 to as high as 1100 per million were observed in 8 of 12 vaccinees (Table 14). The efficacies of the mCMV- and hCMV- driven nef constructs are comparable on the basis of the data generated thus far.

Table 14. Nef-specific T cell Responses in MRKAd5nef Immunized Rhesus Macagues.

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Vaccine (T=0,4 wks)	Monk #	Р	re	T	=4	T:	=7	T=	:16
		Mock	Nef	Mock	Nef	Mock	Nef	Mock	Nef
MRKAd5hCMV-nef(G2A,LLAA) (E3+)	CD2D	0	4	31	440	4	368	1	251
10^11 vp	CC7B	0	0	2	521	0	178	1	1522
	CC61	2	9	31	112	0	108	11	100
MRKAd5hCMV-nef(G2A,LLAA) (E3+)	CC2K	9	9	6	52	0	35	0	15
10^9 vp	CD15	5	4	30	998	2	586	0	434
	CD16	6	1	6	1146	0	369	1	212
MRKAd5mCMV-nef(G2A,LLAA) (E3+)	99D191	1	5	4	614	0	298	2	419
10^11 vp	99D144	4	6	5	434	0	1100	2	932
	99C193	1	2	1	58	1	22	0	64
MRKAd5mCMV-nef(G2A,LLAA) (E3+)	- 99D224	1.	11	14	_ 231	. 1	125	- 0	70
10^9 vp	99D250	8	9	4	108	0	54	0	5
	99C120	1	6	20	299	0	92	0	79
Naīve	083Q	nd	nď	18	22	4	5	2	1

EXAMPLE 25

15 Comparison of Clade B vs. Clade C T Cell Responses in HIV-Infected Subjects PBMC samples collected from two dozens of patients infected with HIV-1 in US were tested in ELISPOT assays with peptide pools of 20-mer peptides overlapping by 10 amino acids. Four different peptide pools were tested for cross-clade recognition, and they were either derived from a clade B-based isolate (gag H-b; nefb) or a clade C-based isolate (gag H-c, nef-c). Data in Table 15 shows that T cells 20 from these patients presumably infected with clade B HIV-1 could recognize clade C gag and nef antigens in ELISPOT assay. Correlation analysis further demonstrated that these T cell responses against clade C gag peptide pool were about 60% of the clade B counterpart (Figure 24), while the T cell responses against clade C nef were about 85% of the clade B counterpart (Figure 25). These results suggest that cellular 25 immune responses generated in patients infected with clade B HIV-1 can recognize gag and nef antigens derived from clade C HIV-1. These data show that a HIV vaccine, such as a DNA or MRKAd5-based adenoviral vaccine expressing a clade B

gag and/or nef antigen will potentially have the ability to provide a prophylactic and/or therapetic advantage on a global scale.

Table 15
Responses Shown as the Number of gIFN-Secreting T Cells per Million PBMCs

subject	bleed date	gag epitope #	mock	gag H-b	gagH-c	nef-b	nef-c
	(from mapping)					
#100	19-Jul-99	12	10	3950	1385	1295	1300
#101	25-Jul-99	3	15	3885	1280	na	1020
#102	25-Jul-99	4	15	1740	850	1255	1785
#104	7-Jun-99	2	5	1355	1185	na	1060
#107	11-Oct-99	2	25	3305	2795	670	870
#405	11-Jul-99	2	15	4575	3180	1700	1500
#501	19-Jul-99	2	15	1100	570	3365	3460
#505	18-Jul-99	5	10	2145	1725	1235	na
#506	28-Feb-99	2	25	150	45	400	610
#701	28-Mar-99	5	30	7620	4775	3320	2780
#709	17-May-99	3	15	2785	1945	1090	1630
#710	24-May-99	4	5	1055	1080	2210	2140

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EXAMPLE 26 Characterization and Production of MRKAd5pol and MRKAd5nef Vectors in Roller Bottles

Expansion of nef and pol Adenovectors - Nef and pol CsCl purified MRKAd5 seeds were used to infect roller bottles to produce P4 virus to be used as a seed for further experiments. P4 MRKAd5 pol and nef vectors were used to infect roller bottles at an MOI 280 vp/cell, except for hCMV-tpa-nef [E3+] which was infected at an MOI of 125 due to low titers of seed obtained at P4.

Table 16 Viral particle concentrations for P5 nef and pol adenovectors

Adenovector	AEX Titer	AEX Titer	Amplification
	(10 ¹⁰ vp/ml culture)	(10 ⁴ vp/cell)	Ratio
hCMV-FL-nef [E3+]	1.1	0.9	. 30
mCMV-FL-nef [E3+]	2.2	2.1	75
hCMV-tpa-nef [E3+]	0.07	0.1	5
mCMV-tpa-nef [E3+]	1.3	0.9	35
hCMV-FL-pol [E3+]	2.7	2.1	75
hCMV-FL-pol [E3-]	1.9	1.3	45

5 Roller Bottle Passaging - Passaging of the pol and nef constructs continued through passage seven. Cell-associated (freeze/thaw lysis) and whole broth (tritonlysis) titers obtained in all passages were very consistent. In general, MRKAd5pol is ca. 70% as productive as MRKAd5gag while MRKAd5nef is ca. 25% as productive as MRKAd5gag. Samples of P7 virus for both constructs were analyzed by V&CB by restriction digest analysis and did not show any rearrangements.

Table 17. Passage Six Viral Productivity for MRKAd5pol and MRKAd5nef

			0° cells/ml), ity (%) Harvest	Cell Passage Number	AEX Titer (Cell Associated) 10 ¹⁰ vp/ml culture	Titer 10 ⁴ vp/cell	Amplification Ratio	Triton Lysis Titer 10 ¹⁰ vp/ml culture
hCMV-FL-nef [B3+]	pool	1.22, 85%	122.103.	62	0.8	0.7	25	1.6
	1 2		0.99, 62% 1.10, 72%					
bCMV-FL-pol [E3+]	pool	1.42, 89%		62	4.5	3.2	115	7.0
	2		1.22, 70% 1.42, 74%					

15 Table 18. Passage Seven Viral Productivity for MRKAd5pol and MRKAd5nef

			0 ⁶ cells/ml), ity (%) Harvest	Cell Passage Number	AEX Titer (Cell Associated) 10 ⁷⁰ vp/ml culture	Titer 10 ⁴ vp/cell	Amplification Ratio	Triton Lysis Titer 10 ¹⁰ vp/ml culture
hCMV-FL-nef [E3+]	Pool	1.33, 90%		66	1.0	0.8	29	2.1
	1		0.96, 70%					
	2		1.18, 73%	.}	}	İ		
bCMV-FL-pol [E3+]	Pool	0.90*, 90%		56	4.2	4.7	168	6.5
	1		1.18, 88%		· · · · · · · · · · · · · · · · · · ·			
	2		1.04, 80%					

MRKAd5nef and MRKAd5pol Viral Production Kinetics - A timecourse experiment was carried out in roller bottles to determine if the viral production kinetics of the MRKAd5pol and MRKAd5nef vectors were similar to those of MRKAd5gag. PER.C6® cells in roller bottle cultures were infected at an MOI of 280 vp/cells with P5 MRKAd5pol, P5 MRKAd5nef and P7 MRKAd5gag; for each adenovector, two infected bottles were sampled at 24, 36, 48, and 60 hours post infection. In addition, two bottles were left unsampled until 48 hpi when they were harvested under the Phase I process conditions. The anion-exchange HPLC viral particle concentrations of the freeze-thaw recovered cell associated virus at the 24, 36,

48, and 60 hpi timepoints are shown in Figure 29A-B. The QPA titers show a similar trend (data not shown).

Comparison of hCMV- and mCMV-FL-nef - As the titers obtained with the MRKAd5nef construct (hCMV-FL-nef) were lower than those obtained with MRKAd5gag or MRKAd5pol, a viral productivity comparison experiment was performed with mCMV-FL-nef. For each of the two adenovectors (hCMV- and mCMV-FL-nef), two roller bottles were infected at an MOI of 280 vp/cell with passage five clarified lysate. The macroscopic and microscopic observations of the four roller bottles were identical at the time of harvest. Analysis of the clarified lysate produced indicated a higher viral particle concentration in the bottles infected with mCMV-FL-nef, as shown in Table 19. It is stipulated that the higher productivity with mCMV promoter driven nef vector is due to lower nef expression levels in PER.C6® cells- experiments are underway at V&CB to measure nef expression levels.

Table 19. Passage Six Viral Productivity Comparison of hCMV- and mCMV-FL-nef

	[Xv (10 ⁶ cells/m	l), Viability (%)	Cell Passage	AEX Titer	Titer	Amplification	Triton Lysis Titer
•		Infection	Harvest	Number	10 ¹⁰ vp/ml culture	10 ⁴ vp/cell	Ratio	10 ¹⁰ vp/ml culture
hCMV-FL-nef	Pool	1.11, 91%		60	1.5	1.4	50	2.8
(MRKAd5nef)	1		1.23,75%					
	2		1.34, 74%					
mCMV-FL-nef	Pool	1.11, 91%		60	2.3	2.1	75	4.6
	1		1.49, 84%					
	2		1.18, 77%					_

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EXAMPLE 27

Characterization and Large Scale Production of MRKAd5nef Virus in Bioreactors

Materials and Methods - The experiment of the present example was run twice under the following conditions: 36.5°C, DO 30%, pH 7.30, 150rpm agitation rate, no sparging, Life Technologies (Gibco, Invitrogen) 293 SFM II (with 6mM L-glutamine), 0.5M NaOH as base for pH control. During the first run (B20010115), two 10L stirred vessel bioreactors were inoculated with PER.C6® cells at a concentration of 0.2x106 cells/ml. Cells were grown until they reached a cell concentration of approximately 1x106 cells/ml. The cells were infected with uncloned MRKAd5nef (G2A,LLAA) at a MOI of 280 virus particles (vp)/cell. For the second batch (B20010202), the same procedure as the first run was used, except the cells

were infected with cloned MRAd5nef. During both runs, the bioreactors were harvested 48 hours post-infection. Samples were taken and virus concentrations were determined from whole broth (with triton lysis), supernatant, and cell pellets (3 X freeze/thaw) with the AEX and QPA assays. Metabolites were measured with BioProfile 250 throughout the process.

Table 20: Experimental Conditions

Temperature	36.5 ℃	
DO	30%	
PH	7.30	
Agitation	150 rpm	
Sparging	None	•

Table 21: Virus source used for experiments.

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Run	Batch ID	Cloned/Uncloned MRKAd5nef	MOI (vp/cells)
#1	B20010115-1	Uncloned	280
	B20010115-2	Uncloned	280
#2	B20010202-1	Cloned	280
ì	B20010202-2	Cloned	280

Results - Table 22 and 23 show an the ability to scale up production of MRKAd5nef by growth in a bioreactor.

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Table 22: Virus Concentration as measured by the AEX assay

Run	Batch ID	Cloned/Uncloned	V	irus Concentration @	₱ 48hpi (1x	10 ¹³ vp/L)
		MRKAd5nef	Supernatant	Clarified Lysate	Total	Triton Lysate
#1	B20010115-1	Uncloned	0.72	3.26	3.98	5.76
	B20010115-2	Uncloned	0.38	1.67	2.05	2.46
#2	B20010202-1	Cloned	0.80	6.00	6.80	8.88
	B20010202-2	Cloned	0.50	6.00	6.50	8.47

Table 23: Virus Titers as measured by the OPA assay

Run	Batch ID	Cloned/Uncloned		Virus Concent	ration @ 48hpi	(1x10 ¹¹ IU/L)	
		MRKAd5nef	Whole	Supernatant	Clarified	Total	Triton
			Broth		Lysate		Lysate
#1	B20010115-1	Uncloned	0.13	1.12	1.76	2.88	11.28
	B20010115-2	Uncloned	0.14	0.73	1.54	2.27	5.86
#2	B20010202-1	Cloned	0.14	0.97	1.62	2.69	11.89
	B20010202-2	Cloned	0.14	1.17	1.70	2.97	12.47

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The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art

from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

EXAMPLE 28

MRKAd5HIV-1gag Boosting of DNA-Primed Animals

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Groups of 3-5 rhesus macaques were immunized with (a) 5 mgs of V1Jns-Flgag (pVIJnsCMV(no intron)-FL-gag-bGHpA), (b) 5 mgs of V1Jns-Flgag formulated with 45 mgs of a non-ionic block copolymer CRL1005, or (c) 5 mgs of V1Jns-Flgag formulated with 7.5 mgs of CRL1005 and 0.6 mM benzalkonium chloride at weeks 0, 4, and 8. All animals received a single dose of 10e7 viral particles (vp) of the MRKAd5HIV-1gag at week 26. Note: 10e7 is too low to prime or boost effectively when used as a single modality (dose is selected to mimic preexposure to adenovirus); see Figure 32.

Blood samples were collected from all animals at several time points and peripheral blood mononuclear cells (PBMCs) were prepared using standard Ficoll method. The PBMCs were counted and analyzed for gamma-interferon secretion using the ELISpot assay (Table 24). For each monkey, the PBMCs were incubated overnight either in the absence (medium) or presence of a pool (called "gag H") of 50 20-aa long peptides that encompass the entire HTV-1 gag sequence.

The results indicate that MRKAd5HIV-1gag was very effective in boosting the T cell immune responses in these monkeys. At week 28 or 2 weeks after the viral boost, the number of gag-specific T cells per million PBMCs increased 2-48 fold compared to the levels observed at week 24 or 2 weeks prior to the boost.

The PBMCs were also analyzed by intracellular gamma-interferon staining prior to (at week 10) and after the MRKAd5gag boost (at week 30). The results for select animals are shown on Figure 31. The results indicate that (a) immunization with DNA/adjuvant formulation elicited T cell responses which can either be balanced, CD4⁺-biased or CD8⁺-biased, and (b) boosting with the MRKAd5gag construct produced in all cases a strongly CD8⁺-biased response. These results suggest that boosting with MRKAd5HIV-1gag construct is able to improve the levels of antigen-specific CD8⁺ T cells.

Table 24. Boosting of DNA/Adjuvant-Primed Rhesus Monkeys with MRKAd5gag Number of SFC/million PBMCs

MRKAdSgap[E3+] CB5H Medium gag n Medium gag H Medium	Ē.	Priming T=0.4 B wife	Ton Work	-	۲ŀ				# -	ı.	T=10	T=17	17	T=24	4	۲	ייאו	T=28	H
MFKAdSpag(E3+) CBSH NA NA 3 35 15 71 4 224 8 115 6 16 16 16 170 7	ſ.	THE O MAIN		Medium	4	4		Medium	4	Medium	gan H	Wedlum	980 14	Wedlum	gag H	Medium	_	_	_
MPRKAdSpag(E3+) CC1C 0 0 15 11 0 0 35 1 3 61 3 48 0 75 89 8 65 65 11 0 0 35 1 1 0 0 35 1 1 0 0 35 1 1 0 0 35 1 1 0 0 35 1 1 0 0 35 1 1 0 0 35 1 1 0 0 35 1 1 0 0 35 1 1 0 0 35 1 1 0 0 35 1 1 0 0 35 1 1 0 0 0 35 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	_	DNAVS mgs		¥ Z	¥	_	R	12	7	7	524	В	115	9	92	200		929	956 0
MPKAdōgag(E3+) CC1C 0 4 1 60 0 111		Peg		<u>-</u>	0	_	5	0	46	٥	89	0	76	0	æ	6		1706	
MPKAdspag(E3+) CC1C 0 4 1 60 0 111 5 270 4 280 8 232 3 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1		(10101)	AW3G	6	=		8	რ 	5	8	48	N	88	80	8	DL .		686	0 688
MRNAddgagl(E3+) AW20 10 4 1 1 50 0 11 1 150 0 284 19 425 0 321 3 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	6	DNA/Emas		ļ.	ŀ	ŀ	ļ	1	ļ								- 1	1	4
MPKAdsgrapt[E3+] AW20 10 4 1 101 0 254 0 791 5 452 0 321 0 321 0 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6	,			·	•	-	3	-	<u>=</u>	٥	270	4	280	m	25	0	8	20	_
AW3P 9 8 1 1 10 4 71 4 164 8 104 6 6 85 11		CHL1003/45mgs		4	-	-	<u>-</u>	0	264	0	791	25	452	0	32	0	<u>ē</u>	<u>_</u>	_
MRKAdSpag(E3+) AWZO 10 4 1 59 5 19 19 574 9 251 8 4 19 1077vp CBSF 8 6 0 6 6 10 26 19 19 10 10 10 10 10 10 10 10 10 10 10 10 10			AW3P	6	80	-	₽	*	_	4	154	60	\$	20	路	=	8	_	_
MRKAdsgrapt[E3+] AWZO 10 4 1 1 59 5 264 19 425 6 105 9 205 18 14 10×7 vp CBs8 8 6 0 6 6 3 119 0 270 5 130 1 1 105 14 CGsW 4 3 0 28 1 9 0 16 1 609 5 625 1 769 0 1 1 105 14 105 14 1 105 14 1 105 14 1 105 14 1 105 14 1 105 14 1 105 14 1 105 14 1 105 14 1 105 14 1 105 14 1 105 14 1 105 14 1 105 14 1 105			CBSF	Š	₹	<u>-</u>	౯	• -	88	0	230	6	374	6	261	80	20	_	23
MRRAddengite3+1 AW20 10 4 1 59 5 264 19 425 6 105 9 205 18 10 10 10 10 10 10 10 10 10 10 10 10 10			AKBB	6	5	₹.	8	<u>-</u>	119	•	439	0	425	0	316	4	1228	_	
1077 p CAAR 1 0 3 121 1 135 1 270 5 130 1 105 14 CBS 8 6 0 6 7 3 119 0 274 6 282 1 208 0 CBY 4 3 0 26 1 9 0 138 0 164 1 62 6 CBY 1 0 0 136 0 316 1 609 5 626 1 769 0	6	DNA/5 mgs+		٥		<u> </u> -	23	2	264	19	425	9	5	6	205	202	585	+-	8
CBS		CRL1005/7.5 mgs + 0.9 mM BAK		-	0	e	12	<u>-</u>	135	-	22	r)	윤	_	50	=	1384	_	2
CBTO 1 0 0 28 1 91 0 139 0 164 1 62 5 CBTO 1 1 0 0 1316 1 609 5 625 1 759 0 CBTO 1 1 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0			CB28	6	9	•	9	eo	119	0	274	8	282	-	208	•	838	_	-
CBTO 1 0 0 136 1 609 5 625 1 759 0			CB6W	4	e	<u> </u>	83	_	6	Ö	139	0	\$	_	8	50	643	_	-
None 980201 3 0 0 0 1 0 0 0 1			CB70	-	6	<u> </u>	136	o 	316	,	66	ω	979	-	769	•	2278		•
	4	none	None 98D201		0	0	0	-	•	٥	٥	ŀ	-	-	~	6			-

EXAMPLE 29

Construction of gagpol fusion for MRKAd5gagpol fusion constructs

The open reading frames for the codon-optimized HIV-1 gag gene was fused directly to the open reading frame of the IA pol gene (consisting of RT, RNAseH and integrase domains) by stepwise PCR. Because the gene (SEQ ID NO: 38) does not include the protease gene and the frameshift sequence, it encodes a single polypeptide of the combined size of p55, RT, RNAse H and integrase (1350 amino acids; SEQ ID NO: 39).

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The fragment that extends from the BstEII site within the gag gene to the last non-stop codon was ligated via PCR to a fragment that extends from the start codon of the IApol to a unique BamHI site. This fragment was digested with BstEII and BamHI. Construction of gag-IApol fusion was achieved via three-fragment ligation involving the PstI-BstEII gag digestion fragment, the BstEII/BamHI digested PCR product and long PstI/BamHI V1R-FLpol backbone fragment.

The MRKAd5-gagpol adenovirus vector was constructed using the BglII fragment of the V1R-gagpol containing the entire ORF of gag-IApol fusion gene.

EXAMPLE 30

Immunogenicity Studies in Non-Human Primates

Cohorts of three (3) macaques were immunized with 10e8 or 10e10 viral particles (vp) of one of the following MRKAd5 HIV-1 vaccines: (1) MRKAd5gag; (2) MRKAd5pol; (3) MRKAd5nef; (4) a mixture containing equal amounts of MRKAd5gag, MRKAd5pol, and MRKAd5nef, or (5) a mixture of equal amounts of MRKAd5gagpol and MRKAd5nef. The vaccines were administered at weeks 0 and 4.

The T cell responses against each of the HIV-1 antigens were assayed by IFN-gamma ELISpot assay using pools of 20-aa peptides that encompass the entire protein sequence of each antigen. The results (Table 25) are expressed as the number of spot-forming cells (sfc) per million peripheral blood mononuclear cells (PBMC) that respond to each of the peptide pools.

Results indicate the following observations: (1) each of the single gene constructs (MRKAd5gag, MRKAd5pol, or MRKAd5nef) is able to elicit high levels of antigen-specific T cells in monkeys; (2) the single-gene MRKAd5 constructs can be mixed as a multi-cocktail formulation capable of eliciting very broad T cell responses against gag, pol, and nef; (3) the MRKAd5 vector expressing the fusion

protein of gag plus IA pol is capable of inducing strong T cell responses to both gag and pol.

Table 25. Evaluation of Mixtures of MRKAd5 vectors expressing humanized

5 HIV-1 gag, pol, gagpol, nef in rhesus macaques

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Grp#	- Vaccine	Monk #			T=6 wks		
	T=0, 4 wks	l i	Mock	Gag H	Pol - 1	Pol - 2	Nef
1	MRKAd5 gag	CB9V	0	15	-	-	-
	10^10 vp	CD19	0.	374	-	-	-
		109H	1	843	-	-	-
2	MRKAd5 gag	99D130	1	948	-	- 1	-
	10^8 vp	W277	16	324	-	-	-
		143H	. 4	595	-	-	•
3	MRKAd5 pol	CC1X	4	-	46	256	-
	10^10 vp	AW3W	3	-	463	550	-
İ		AV43	6	-	95	1333	-
4	MRKAd5 pol	AW38	1	-	19	30	-
ļ	10^8 vp	CC8K	0	-	50	995	-
		CC21	1	-	33	· 436	-
5	MRKAd5 nef	076Q	9	-	-	-	1204
	10^10 vp	091Q	4		-	-	85
		083Q	0	-	-	-	176
6	MRKAd5 nef	00C029	1	-	-	-	114
	10^8 vp	98D022	6	-	-	-	170
		98D160	3	-	-	-	198
7	MRKAd5gag+MRKAd5pol+MRKAd5nef	99D251	3	206	15	193	120
	10^10 vp each	05H	3	135	21	9	638
ļ	·	00C016	3	26	4	51	23
8	MRKAd5gag+MRKAd5pol+MRKAd5nef	99D215	1	171	18	193	240
	10^8 vp each	81H	5	73	6	14	243
		-12H	8	1140	115	811	719
9	MRKAd5gagpol +MRKAd5 nef	99D211	0	83	56	838	725
	10^10 vp each	22H	4	385	119	1194	1915
		61H	4	343	11	765	853
10	MRKAd5gagpol +MRKAd5 nef	34H	3	78	19	5	75
ĺ	10 ⁸ vp each	48H	1	65	105	46	43
ľ		70H	5	158	15	220	191

Indicated are numbers of spot-forming cells per million PBMCS against the peptide pools. Mock, no peptides; gag H, fifty 20-aa peptides encompassing p55 sequence; pol-1, 20-aa peptides representing N-terminal half of IA pol; pol-2, 20-aa peptides representing the carboxy-terminal half of IA pol; nef, 20-aa peptides encompassing the entire wild-type nef sequence. Responses to the antigens prior to the first immunization did not exceed 40 sfc/10^6 PBMC.

WHAT IS CLAIMED IS

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1. A recombinant adenoviral vaccine vector at least partially deleted in

- 5 E1 and devoid of E1 activity, comprising:
 - a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to between from about base pair 400 to about base pair 458 of a wildtype adenovirus genome; and
 - b) a gene encoding an HIV protein or immunologically relevant modification thereof.
 - A vector in accordance with claim 1 comprising a packaging region corresponding to from about base pair 1 to about base pair 450 of a wildtype adenovirus genome.
- 3. A vector in accordance with claim 1 further comprising nucleotides
 15 corresponding to between from about base pair 3511 to about 3524 to about base pair
 5798 of a wildtype adenovirus genome.
 - A vector in accordance with claim 3 comprising base pairs corresponding to 1-450 and 3511-5798 of a wildtype adenovirus genome.
- 5. A vector in accordance with claim 4 which is deleted of base pairs451-3510.
 - 6. A vector in accordance with claim 1 which is at least partially deleted in E3.
 - 7. A vector in accordance with claim 6 wherein the E3 deleted region is from base pairs 28,133-30,818.

8. A vector in accordance with claim 1 wherein the gene encoding the HIV protein or modification thereof comprises codons optimized for expression in a human.

- 9. A vector in accordance with claim 1 wherein the vector comprises a5 gene expression cassette comprising:
 - a) a nucleic acid encoding a protein;
 - b) a heterologous promoter operatively linked to the nucleic acid encoding the protein; and
 - (c) a transcription termination sequence.
- 10. A vector in accordance with claim 9 wherein the gene expression cassette is inserted into the E1 region.
 - 11. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette is in an E1 parallel orientation
- 12. An adenoviral vector in accordance with claim 9 wherein the geneexpression cassette is in an E1 antiparallel orientation.
 - 13. An adenoviral vector in accordance with claim 9 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.
 - 14. An adenoviral vector in accordance with claim 13 wherein the promoter is an immediate early human cytomegalovirus promoter.
- 20 15. An adenoviral vector in accordance with claim 9 wherein the promoter is a murine cytomegalovirus promoter.
 - 16. An adenoviral vector in accordance with claim 9 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

17. An adenoviral vector in accordance with claim 9 wherein the transcription termination sequence is a synthetic polyadenylation signal (SPA).

- 18. A cell comprising the adenoviral vector of claim 1.
- 19. Recombinant, replication-defective adenovirus particles harvested
 and purified subsequent to transfection of the adenoviral vector of claim 1 into a cell
 line which expresses adenovirus E1 protein at complementing levels.
 - 20. An HIV vaccine composition comprising purified adenovirus particles of claim 19.
- 21. An HIV vaccine composition of claim 20 which comprises aphysiologically acceptable carrier.
 - 22. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 1 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.
 - 23. A method according to claim 22 wherein the cell is a PER.C6® cell.

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- 24. A method of generating a cellular-mediated immune response
 against HIV in an individual comprising administering to the individual a vaccine of
 claim 21.
 - 25. A method according to claim 24 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

26. A method according to claim 25 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

- 27. A method according to claim 24 wherein the adenovirus vaccine is
 5 preceded by an adenovirus vaccine of a different serotype.
 - 28. A method according to claim 24 which comprises administering and readministering the adenovirus vaccine vector to the individual.
 - 29. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV gag or an immunologically relevant modification thereof.
- 30. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV gag protein or immunologically relevant modification thereof.
 - 31. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:
- a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and
- 20 b) a gene expression cassette comprising
 - i) SEQ ID NO: 29;
 - ii) a heterologous promoter operatively linked to i); and
 - iii) a transcription termination sequence.

32. An adenoviral vector in accordance with claim 31 wherein the gene expression cassette is in an E1 parallel orientation.

- 33 An adenoviral vector in accordance with claim 31 wherein the gene expression cassette is in an E1 antiparallel orientation.
- 34. An adenoviral vector in accordance with claim 31 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

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- 35. An adenoviral vector in accordance with claim 31 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.
- 36. An adenoviral vector in accordance with claim 31 which is at least partially deleted in E3.
 - 37. A cell comprising the adenoviral vector of claim 30.
- 38. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 30 into a cell line which expresses adenovirus E1 protein at complementing levels.
- 39. An HTV vaccine composition comprising purified adenovirus particles of claim 38.
- 40. An HIV vaccine composition of claim 39 which comprises a physiologically acceptable carrier.
- 41. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 30 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

42. A method according to claim 41 wherein the cell is a PER.C6® cell.

43. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 21.

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- 44. A method according to claim 43 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.
- 45. A method according to claim 44 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.
 - 46. A method according to claim 43 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.
- 47. A method according to claim 43 which comprises administering and readministering the adenovirus vaccine vector to the individual.
 - 48. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV pol or an immunologically relevant modification thereof.
- 49. An adenoviral vector in accordance with claim 9 wherein the gene
 20 expression cassette comprises an open reading frame encoding an HIV pol protein or immunologically relevant modification thereof.
 - 50. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and

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- b) a gene expression cassette comprising
 - i) a nucleotide sequence selected the group consisting of SEQ ID NO: 1, SEQ ID NO: 5 and SEQ ID NO: 7;
 - ii) a heterologous promoter operatively linked to i); and

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- iii) a transcription termination sequence.
- 51. An adenoviral vector in accordance with claim 50 wherein the gene expression cassette is in an E1 parallel orientation.
- 52. An adenoviral vector in accordance with claim 50 wherein the gene expression cassette is in an E1 antiparallel orientation.

53. An adenoviral vector in accordance with claim 50 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

- 54. An adenoviral vector in accordance with claim 50 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.
- 55. An adenoviral vector in accordance with claim 50 which is at least partially deleted in E3.
 - 56. A cell comprising the adenoviral vector of claim 49.

57. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 49 into a cell line which expresses adenovirus E1 protein at complementing levels.

58. An HIV vaccine composition comprising purified adenovirus particles of claim 57.

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- 59. An HIV vaccine composition of claim 58 which comprises a physiologically acceptable carrier.
- 60. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 49 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.
- 61. A method according to claim 60 wherein the cell is a PER.C6® cell.
- 15 62. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 59.
 - 63. A method according to claim 62 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.
 - 64. A method according to claim 63 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

65. A method according to claim 62 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

- 66. A method according to claim 62 which comprises administering and readministering the adenovirus vaccine vector to the individual.
- 67. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV nef or an immunologically relevant modification thereof.

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- 68. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV nef protein or immunologically relevant modification thereof.
- 10 69. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:
 - a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and
 - b) a gene expression cassette comprising
 - a nucleotide sequence selected the group consisting of SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13 and SEQ ID NO: 15;
 - ii) a heterologous promoter operatively linked to i); and
 - iii) a transcription termination sequence.
 - 70. An adenoviral vector in accordance with claim 69 wherein the gene expression cassette is in an E1 parallel orientation.

71. An adenoviral vector in accordance with claim 69 wherein the gene expression cassette is in an E1 antiparallel orientation.

- 72. An adenoviral vector in accordance with claim 69 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.
- 5 73. An adenoviral vector in accordance with claim 69 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.
 - 74. An adenoviral vector in accordance with claim 69 which is at least partially deleted in E3.
 - 75. A cell comprising the adenoviral vector of claim 68.

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- 76. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 68 into a cell line which expresses adenovirus E1 protein at complementing levels.
- 77. An HIV vaccine composition comprising purified adenovirus particles of claim 76.
 - 78. An HIV vaccine composition of claim 77 which comprises a physiologically acceptable carrier.
 - 79. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 68 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.
 - 80. A method according to claim 79 wherein the cell is a PER.C6® cell.

81. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 78.

- 82. A method according to claim 81 which further comprises

 administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.
 - 83. A method according to claim 82 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

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- 84. A method according to claim 81 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.
- 85. A method according to claim 81 which comprises administering and readministering the adenovirus vaccine vector to the individual.
- 86. A multivalent adenovirus vaccine composition comprising recombinant, replication-defective adenovirus particles, wherein the adenovirus particles are harvested and purified from a cell line expressing adenovirus E1 protein, and wherein the particles are harvested subsequent to transfection of the cells with an adenoviral vector or vectors in accordance with claim 9; said vector(s) comprising a gene expression cassette or cassettes comprising nucleotide sequences encoding HIV proteins selected from the group consisting of:
 - gag, pol, and nef, expressed independently from three individual vectors;

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b) gag, pol, and nef, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences; c) gag, pol, and nef, expressed via two vectors, one expressing a pol-. 5 nef fusion, and another expressing gag; d) gag, pol, and nef, expressed via two vectors, one expressing a gagpol fusion and another expressing nef; e) gag, pol and nef, expressed via two vectors, one expressing a nefgag fusion and another expressing pol; 10 gag, pol, and nef, expressed via one vector expressing a gag-polnef fusion; gag and pol, expressed independently from two individual vectors; h) gag and pol, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct 15 promoters and transcription termination sequences; i) pol and nef, expressed independently from two individual vectors; pol and nef, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences; 20 k) nef and gag, expressed independently from two individual vectors; nef and gag, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences; m) gag and pol, expressed via one vector expressing a gag-pol fusion;

n) pol and nef, expressed via one vector expressing a pol-nef fusion; and

- o) nef and gag, expressed via one vector expressing a nef-gag fusion.
- 87. A multivalent adenovirus vaccine composition in accordance with claim 86 wherein the gag-pol fusion consists of SEQ ID NO: 39.
 - 88. A multivalent adenovirus vaccine composition in accordance with claim 86 wherein the fused sequences have the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences.
- 89. A multivalent adenovirus vaccine composition in accordance with

 10 claim 86 wherein the fused sequences have the encoding nucleic acid sequences

 operatively linked to a single promoter; and the encoding nucleic acid sequences

 operatively linked by an internal ribosome entry sequence ("TRES").

Original Adenovector Construct:

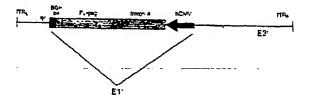


Figure 1: Original HIV-1 gag adenovector.

Sequence of the open reading frame for FL-gag (human codon optimized)

atgggtgctagggcttctgtgctgtctggtggtgagctggacaagtgggagaagatcaggctgaggcctggtgg caagaagaagtacaagctaaagcacattgtgtgggcctccagggagctggagaggtttgctgtgaaccctggc agctgaggtccctgtacaacacagtggctaccctgtactgtgtgcaccagaagattgatgtgaaggacaccaag gaggccctggagaagattgaggaggagcagaacaagtccaagaagaaggcccagcaggctgctgctggc acaggcaactccagccaggtgtcccagaactaccccattgtgcagaacctccagggccagatggtgcaccag gccatctcccccggaccctgaatgcctgggtgaaggtggtggaggagaaggccttctcccctgaggtgatccc catgitictetgecetgictgagggtgecacceceaggacetgaacaccatgetgaacacagtggggggecate aggetgecatgeagatgetgaaggagaceateaatgaggaggetgetgagtgggacaggetgeateetgtge acgetggccccattgcccccggccagatgagggagcccaggggctctgacattgctggcaccacctccaccct ccaggagcagattggctggatgaccaaccaccccccatccttgtgggggaaatctacaagaggtggatcat cccttcagggactatgtggacaggttctacaagaccctgagggctgagcaggcctcccaggaggtgaagaact ggatgacagagaccctgctggtgcagaatgccaaccctgactgcaagaccatcctgaaggccctgggccctg ctgccaccctggaggagatgatgacagcctgccagggggtggggggccctggtcacaaggccagggtgctg gctgaggccatgtcccaggtgaccaactccgccaccatcatgatgcagaggggcaacttcaggaaccagag gaagacagtgaagtgcttcaactgtggcaaggtgggccacattgccaagaactgtagggcccccaggaaga ggcaaaatctggccctcccacaagggcaggcctggcaacttcctccagtccaggcctgagcccacagcccct agetglaccccetggcctccetgaggtccctgtttggcaacgacccctcctcccagtaaaataaagcccgggca gat (SEQ ID NO: 29)

Figure 2

Old Transgene:



New Transgenes:

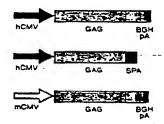


Figure 3: Diagrammatic representation of the original HIV-1 gag transgene and the series of new transgene constructions.

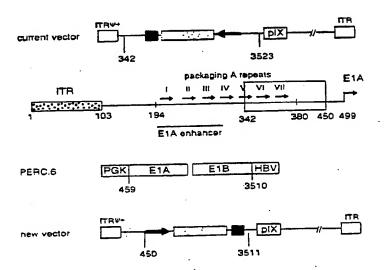


Figure 4: Modifications made to the current adenovector backbone in the generation of the new vector.

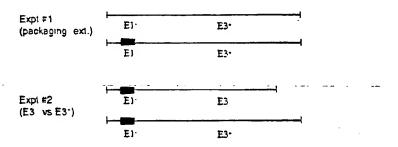


Figure 5: Virus mixing experiments to determine the effects of the addition made to the packaging signal region (Expt #1) and analysis of the effects of the E3 gene on viral growth (Expt. #2). The red bars denote the region of modifications made to the E1 deletion.

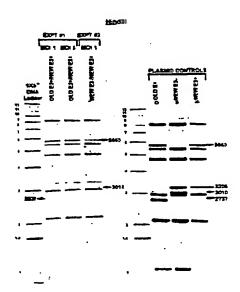


Figure 6: Autoradiograph of viral DNA analysis following viral mixing experiments (expts. #1 and #2) as detailed in the text.

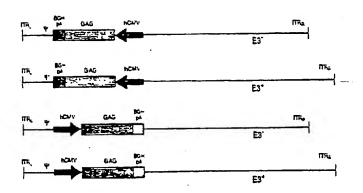


Figure 7A: hCMV-FLgag-bGHpA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

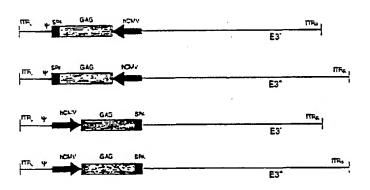


Figure 7B: hCMV-FLgag-SPA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

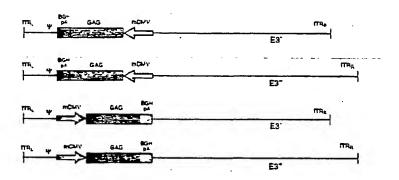


Figure 7C: mCMV-FLgag-bGHpA adenovectors constructed within the *MRK' backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

Plasmid mixing expt: (orientation)

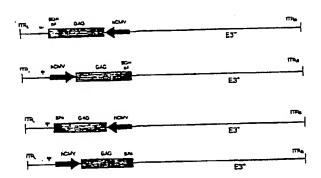


Figure 8A: Effect of transgene orientation

Plasmid Mixing expt: (poly A signal)

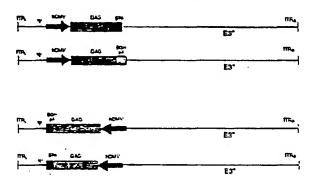


Figure 8B: Effect of polyadenylation signal

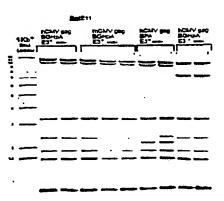


Figure 9: Viral DNA from the four Adgag candidates at P5, following EstE11 digestion.

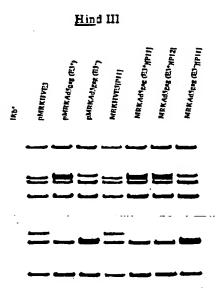


Figure 10: Viral DNA analysis of passage 11 and/or 12 of MRKHVE3, MRKAd5gag and MRKAd5gag(E3-).

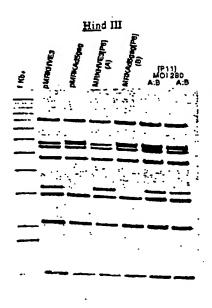


Figure 11: Viral DNA analysis (*Hin*dIII digestion) of passage 6 MRKHVE3 and MRKAd5gag used to initiate the viral competition study. Last two lanes are passage 11 analysis of duplicate passages of the competition study (each virus at MOI 280 vp).

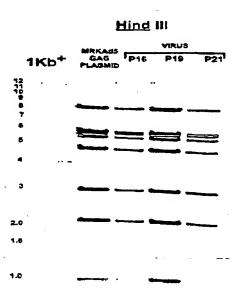
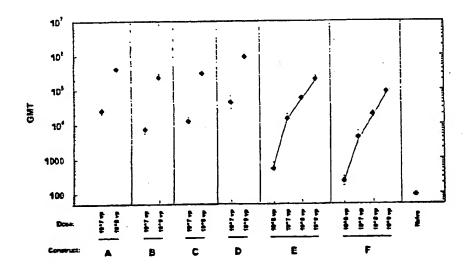


Figure 12: Viral DNA analysis by *HindIII* digestion on high passage numbers for MRKAd5gag in serum containing media with collections made at specified times. The first lane shows the 1 Kb DNA size marker. The other lanes represent pre-plasmid control (digested with Pac1 and *HindIII*), and MRKAd5gag virus continually passaged to P16, P19 and P21(serum containing media).

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Figure . Serum anti-p24 Levels at 3 Wks post i.m. immunization of balb'c mice (n=10) with Varying Doses of Several Adgag constructs: (A) MRKAd5gag (through passage 5): (B) MRKAd5 E3* hCMV-FLgag-bGHpA; (C) MRKAd5 E3* hCMV-FLgag-SPA; (D) MRKAd5 E3* mCMV-FLgag-bGHpA; (D) research Lot (293 cell-derived) of Ad5HIV-lgag; and (F) clinical lot (Ad5gagFN0001) of Ad5HIV-lgag. Reported are the geometric mean titers (GMT) for each cohort.



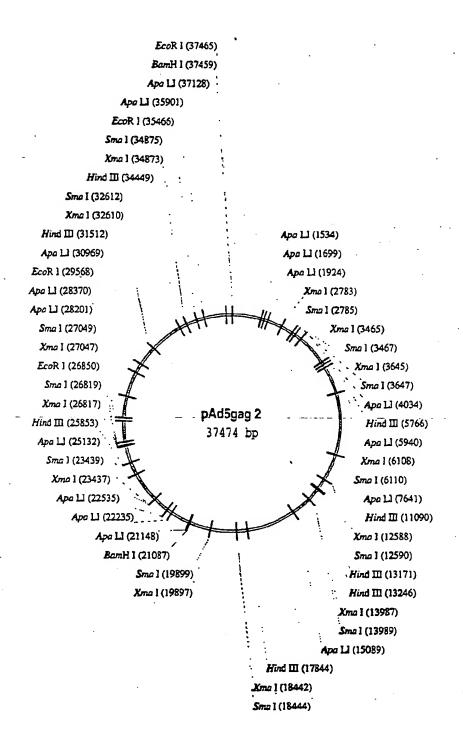


Figure 14

-	- PTCTTAATTA	ACATCATCAA	TAATATACCT	TATTTRICAT	TRANSPORT					CHCCCAACGG
	AACAATTAAT	TCTAGTAGTT	ATTATATAGA	ATAAAACCTA	ACTES CRETE'A		CLCCCACCTC	MACACTGCA	ככביניורניכנו	בעררניוניו
101	CACCAGGTOAC	GTAGTAGNGT	เลตตลางการ	TGATGTTVICA	ACTITITION		ACCESACCIONT	CHCCCAMAG	TCACCTTTTT	פנווגיוטנינייי.
	CCCCCACTG	CATCATCACA	CCCCCTTCAC	ACTACAACGT	ויכאכאככסביני		TCGCTCCTA	בארניפווויור	רופרשמים	CCNC)**********************************
201	OGTGTACACA	GOAAGTGACA	ATTTRIBUTE	CCFFFFAGGC	กาลาสาราชาก		CCCATACCGA	GTAACATTTG	CCCATTITICG	CCCCANAN T
	CCACATOTOT	CCTTCACTGT	TWANACTOR	CCANANTCCG	CULTACAACAT		COCAT ICANO		COMPRECING	The Carry of the C
301	GNATAAGAGG	NACTONNATO	TCAATAATT	TOTOTACTO	ATACICICITA		MCA CALLIA GO	GOAL PICAL	CCAAATTCCAC	CHEMICAL C.
	CHIAMETEC	TTCACTTTAG	ACTTATTAAA	ACACAATGAG	TATICACAT		אכנויייניניני	ררוכיייייי	240000000000000000000000000000000000000	Contractor of the Contractor o
401	CAGGIGITIT	TereAddIGT	THEODOGIT	CCOGGTCAAA	CETTYXCCCTTT		פטיטטטטטטט	ATCCATIGCA	TACGITOTAL	CCATAICAL
	GTCCACAAA	AGAGTCCACA	ANACKEGENA	GGCCCAGTTT	CAACCCCAAA	ATAATAATAT	2020022022	TAGGTAACCT	ATGCAACATA	SELATED IN
501	ATATGTACAT	TTATATTGGC	TCATCTCCAA	CATTACCGCC	ATGTTGACAT		CTACTTATTA	ATAGTAATCA	ATTACGGGGT	CATTAGETICA
	TATACATGTA	AATATAACCG		GTAATGGCGG	TACAACTGTA	ACTANTANCT	GATCAATAAT	TATCATTAGT	TAATGCCCCA	GIRATICARGI
601	TAGCCCATAT	ATCGAGITEC	GCGTTACATA	ACTIACGGIA	AATGCCCCC	CTGGCTTGACC	מכככשעכמעכ	CCCCCCCCAT	TGACGTCAAT	AATGACCTAT
	ATCOGGTATA	TACCTCAAGG	_	TCAATGCCAT	TTACCGGGG	GACCGACTEG	cocorracto	GROCCOCOTA	ACTOCAGITIA	TTACTGCATA
701	GITCCCATAG	TAACCCCAAT	AGGGACTITIC	CATTGACGTC	ANTGGGTGA	CTATTTACK	TANACTISCCC	ACTROCCAGE	ACATCAAGTO	TATCATATC
	CAAGGGTATC	ATTOCOGITA		GTAACTGCAG	TTACCCACCT	CATAMATEXIC	ATTTGACGG	TGAACCGTCA	TGTAGTTCAC	ATAGTATACC
108	CAAGTACOCC	CCCTATTGAC	GTCAATCACG	GTAAATGCCC	CCCCTCCCAT	TATGCCCAGT	ACATGACCTT.	ATGGGACTIT	CCTACTTOGC	AGTACATCTA
!	GITCATGCGG	GOCATAACTO	CAGTTACTGC	CATTTACCGG	GCFGACCGTA	ATACGCCTTCA .	TGTACTGGAA	TACCCTGANA	GGATGAACCG	TCATGTAGAT
106	COTATTACTC		CCATCGTGAT	accentimes	CAGFACATCA	ATEGRATERS	ATAGCGCTTT	GACTCACGGG	GATTTCCANG	TCTCACCC.
\ }	GCATAATCAG			CCCANAACC	GTCATUTAGE	TACCCCCACC	TATCCCCANA	CTGAGTGCCC	CTAAAGGTTC	ACAGGTCCC
1001	ATTOMOTOR	ATCAGAGTTT	GTTTTGGCAC	CANAATCAAC	GGGACTITICC	AAAATGTCGT	AACAACTCCO	CCCCATTGAC	CCANATIGGGC	GGTAN/ICGT:
	TAACTGCAGT	-			CCCTGAAAGG	TTTTYACAGCA	THYTHICAGGC	COCCTANCTO	COTITACCCG	CCATCCGCAC
1101	TACCOTCCCA	GCTCTATATA	ACCAGAGCTC	GTTTAGTGAA	CCGTCAGATC	GCCTGGAGAC	GCCATCCACG	CIGITIFICAC	CTCCATAGNA	CACACCCC
	ATGCCACCCT		TCGTCTCGAG	CANATICACTT	GGCAGTCTAG	COGNICATOR	COGTAGGTGC	GACAAAACTO	GAGGTATCTT	Crereactor
							Rgill			
1201	CCGATCCAGC	כובכסכספכב	GGGAACGGTG	CATTGGAACG	CGGATTCCCC		TCACATCTAC			TOCTIGACAGE
	GCCTAOCTCG	-		GTAACCTTGC	GCCTAAGAGG	CACCATTACTO	ACTECTAGATO	GTACCCACGA	TCCCGAAGAC	ACCACACACC
1301	Programme And City	GACAAGTGGG	AGAAGATCAG	פבאמשמטינים	いいしいいいいいいいいいいいいいいいいいいいいいいいいいいいいいいいいいいいい	NGANGTACAA	GCTAAAGCAC		CCTCCAGGGA	GCTKIGAGAGG
	ACCACTCGAC				CCACCGFTCT	TYTHICATEST	CGATTTCGTG	TAACACACCC	OCACCITCCCT	CGACCTUTUC
1401	Triochord	_	_	TCTGAGGGGT	GCAGGCAGAT	CCTGGGCCAR	CICCAGCCCT		AGGCTCTGAG	CACCTCACCT
	AAACGACACT	-		AGACTCCCCA	CGTCCGTCTA	CHENCECCECTE	GAGGTCGGGA		TCCGAGACTC	
1501	CCCTGTACAA	_		CHUTCACCA			CCANGGAGGC	CCTGGAGAAG	ATTONGGAGG	
	COCACATGTT	GTOTCACCGA	TOGGACATGA	-	_				-	
1601	GTCCAAGAAG	-	-			ACCTRUTCCCA	GANCTACCCC	TRACACTOR	ACCICCAGGG TEGAGGTTCC	GGTCTACCAC
	CAGGTTCTTC	Trecedence	שברהארהארה שברה	ACCGTCTCCG	Thanks Trees	TECACAGGG			22.00000	; ; ;

Figure ISA

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1701	CACCAGGCCA	TCTCCCCCCG AGAGGGGGGC	GACCCTTTAAT	CCCTVCCCTCA	ACCORPATIONS AT THE TAXABLE TO	CUTCTICCGG	THETECCETS ANGAGGGGAC	ACCACTAGGG	CATOTTCTCT GTACAAGAGA	GCCCTGTCTG
1001	ACCCACGGTG	CCCCCAGGAC	CTCAACACA GACTTGITAGE	TY:C'TGAAC'AC' ACGAL;TTRITG	ACATT TOTAL	CATCAGGGTG	CCATCCAGAT CCTACGTCTA	GCTGAAGGAG	ACCATCAATG TOGTAGTTAC	ARGARGETTS T
1901	TCAGTGGGAC ACTCACCCTG	AGGCTGCATC TCCGACGTAG	CTCTMCALGE	Tradenceatt Accordata	מכרבלימטבה האמשורבה	ACATKIAGGGA TK:TAC:TCCL:T	CCCCAGGGGCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	TCTGACATTG AGACTGTAAC	CTGCCACCAC GACCGTGGTG	CTCCACCCT!
2001	CAGGAGCAGA	TTGCCTCGAT	GACCIANCIANC	CCCCCATCC	האנאנינינית מאניאנינינית	AATCTACAAG TYAGAYGTTC	ACCACCTAGE TCCACCTAGE	TCCTCGCCCCT	GAACAAGATT	GTGAGGAATGT
2101	ACTCCCCCAC TGAGGGGGTG	CTCCATCCTG	GACATCANGE	ACCCCCCCCT	CCTCGGGGAAG	AGGACTATG TCCCTGATAC	TGGACAGGTT ACCTGTCCAA	CTACAAGACC	CTGAGGGCTG	AGCAGGCCT** TCGTCCGGAS
2201	CCAGGAGGTG	AAGAACTGGA	TGACAGAGAC	CCTGCTGGTG	CAGAATGCCA GTCTTAGGGT	ACCCTGACTG TGGGACTGAC	CANGACCATC	CTGAAGGCCC	TGGGCCCTGC ACCCGGGACG	TGCCACCCTO ACGGTGGGAV
2301	CTCCTCTACT	TGACAGCCTG ACTGTCGGAC	CCAGGGGGTG	GGGGGGCTTG	CHCAGAAGGC	CAGGGTGCTG	GCTGAGGCCA	TCTCCCAGGT ACAGGGTCCA	GACCAACTCC CTGGTTGAGG	GCCACCATC
2401	TGATGCAGAG	GOGCAACTIC	AGGAACCAGA TCCTTGGTCT	GGAAGACAGT	CTTCACGAAG	AACTGTGGGA	AGGTGGGCCA TCCACCCGGT	CATTGCCAAG	AACTGTAGGG TTGACATCCC	CCCCCAAGAA.
2501	GAAGGGCTGC	TOGAAGTGTO	GCNAGGAGGG	CCACCAGATG	AAGGACTGCA	ATCACACCA	GCCCACTTC CCGCTTGAAG	CTCCCCAAAA	TCTGGCCCTC AGACCGGGAG	CCACAAGGG:
2601	AGGCCTOOCA TCCOGACCGT	ACTTCCTCCA TGAAGGAGGT	GTCCAGGCCT	GAGCCCACAG	CCCCTCCCGA	GOAGTECTTIC	AGGITITOCOG TCCAAACCCC	ACCICITICIO	CACCCCAGC	CACMAGCAG
2701	AGCCCATTGA	CAAGGAGCTG	TACCCCCTVIG	CCTCCCTGAG	GTCCCTGTTT	GGCAACGAGG	CCTCCTCCCA	CTAMMTAAA	Bylli GCCCGGGCAG ATCTGCTGTV CGGGCCGGTC TAGACGACA	ATCTGCTGTK:
2801	CCTTCTAGTT		TGTTGTTTGC	CCCTCCCCCC	TECCTTCCTT	GACCCTGGAA	GGTYCCACTC CCACGGTGAG	CCACTGTCCT	TTCCTAATAA	ANTCACOCAN. TTACTCCTT"
2901	TIGCATCOCA	TTGTCTGAGT	AGGTGTCATT TCCAGTAA Pvul	AGGTGTCATF CTATTCTCAGG TCCACAGTAA GATAAGACCC Pval	GCCACCCCAC	GGGGTCCTGT	GCAAGGRASIA CGTTCCCCCT	OCATTOGGAA CCTAACCCTT	GACAATAGCA	GGCATGCTGG CCCTACGACC
3001	GGATOCOGTG CCTACGCCAC	GRATOCOGTO GOCTCTATOO CCTACGCCAC CCGAGATACC		Asch CCGATCGGG CCCGTACTG GGCTAGCGGC GCGCATGAC	AAATCITCITCIC	CCCACCGAAT	AGGGTGGGAA TCCCACCCTT	AGAATATATA TCTTATATAT	AGGTGGGGT TCCACCCCA SPM	CTTATGTAGT GAATACATCA
3101	TTTGTATCTG AAACATAGAC		TTTTGCAGCA GCCGCCCCG ANAACGTCGT CGGCGGCCGCC CAGAATGTCA TAGACTCCAG	CCATGAGGAG GGTACTCGTG CATTGATGGT	CHACTCGTTT GTTTCAGCCAAA	CATCCATCGT CTACCTTCGT TYTCCCCCAAA	TTGTGAGGTC AAGAGTGGAG CTCTAGTAGC	ATMTFTGACA TATAAACTGT TTGACCTACG	ACCICCIATIC TUCCICCIACC	CCCATRACC GGCTACCC**
	GCCCCACGCA				פבטשבאטט		GAGATGATGG	AACTGGATGC	TCTGGCACAG	Acctracege

Figure 15B

CCCGCTTGCA AACACITGCAG GCGCGAACGT TYTTCACGTC GTTTCTCAGC ACCTGTTGAA CAAAGACG TCCACAAA TTTGATTG GATCAAGCAA TATGAATTG GATCAAGCAA			CATATCCCIC CORRINTITY GTATAGGGAG GCCCCTANUT AACTTGGAGA CGCCCTTGTVI TTGAACCTCT GCGGAAACAC		GAAACGSTT TCCGGGGTAG GGGAGATTA CTTTTGCCAA AGGCCCCATC CCCTCTAGTU. Psil ACCGGCTGCA ACTGGTAGTT AAGAGAGCT: TGGCCGACGT TGACCATCAA TTCTCGGAC	CCAAATCCGC CAGAAGGGG TCGCCCCCA GGTTTAGGCG GTCTTCCGCG AGCCGCGGT	GAGCGTTTGA CCAAGGAGTT CCAGGCGGTC CTCGCAACT GGTTCGTCAA GGTCCGCCAT TTCGCTGTA CGCAGTAGT GGTTCGTCGT AAAGCGACAT GCCGTCATCA GCCACGARIA GGGGTGCGCT CCGGGCTGG GGTTGGCCAA GCCCACGGGA GCCCGACGG GGTTGCCCAA
CTTTCTTSAG G GAMMINGTE G ACTTANTOTE F TGANTTACAG C CCAGACTET 3		AOCTTCATGC TCGAAGTACG GGCAGGCCT CCGTCCGGGA	TOTTCCCAGC ACAAGOGTCG TGCGTGGAAG ACGCACCTTC		GGGCGARTIA GAAAGGITT TCCGGGGTAG CCCGCTACIT CITITICCCAA AGGCCCCATC CACACCTATT ACCGGCTGCA ACTGGTAGTT GTGTGGATAA TGGCCGAGGT TGACCATCAA		
ACTICACTETES TEACTCOACA TEACTCOACA ACTEGGGCCCT AAATAAAAAA	CGGGCGAACT GCCAGCAACT		ACCIATEGETA TECAACEGAT TAGAAGGAAA ATCITECETIT	CTYTOGGGAAG OACCCGCTTC TGGGGTATAA AGGCGATATTT	GGGCGATGAA CCCGCTACTT CACACCTATT		
CCTCATTGTG GCTCTVACAC TTGAATTCTT AACCTAAGAA	CCAGCATTRITA	TREAGGTAGG ACCTCCATCG GTAGCAAGCT GATCGTTCGA	CACATATATA CACATAAAAA TCATGTAGCT AGTACATCGA	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	CCCCTAGTAC AGATGACCC CCCCTAGTAC AGATGACCC CAGCCATTAG GCCGTAAAT GTCGGCACC CGGGATTTA	. מארדכתבאדת ו כיוסאטכקדאכ	TECRECATION OFFICERCATE OFFICERCATE CANAGECETE OFFICERCATE CANAGECETE CA
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PSH CCCHTTGTTGTAG CCCHTGGTTCT TCACGGTTCT ACTCTAGGGT TTGTTGGGT	AAGGAACAGGA CGGGGGGGGGT GGGGGGGGGCA	CCATAACICC CCTATTCGGG GTGCCTAAAA CACGGATTTT		AATISATGGGA TTTTACAAAGC AAATGTTTGG	CANCITACC CANCITACC CCACTIACC CCACTIACC	ACTTCGTTAA	AAAAGTTCCCC TCGATCCAGG AGCTAGGTCG
CCCCCCCTTCA GCCCCCTTCA GATCACACT CTACTCTTCA CCCTVAAGGC	GGGACTTCCG AGGGGTTTTG TCCCCAAAAC	AGATACATOG TCTATGTACC GCTGGGGGTG	CATACGTGGG OTATGCACCC ACAGTGTATC	ATTCGTCCAT TAAGCAGGTA ATAGCAGGTA TATCCTGTAA	CACCCTTTGA GTCCGAAACT TGACCAGCTG	CAGGCACCCC	ANGINECTRIC CTREGRITICA ANAIGRITICE CONCORDER TECNICATION OF CANCERCANE TECNITECATE CANTENTE AND ANGINE ANGINE ANGINE CANCERT TECNITECATE CANCERTED ANGINE ANGINE CONCENTRAL ANAMAGINE CENTRECATE ANGINE CENTRECATE ANAMAGINE CENTRECATE ANAMAGINE CENTRECATE ANGINE CENTRECATE ANAMAGINE CENTRECATE CENTRECATE ANAMAGINE CENTRECATE
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TTOORGACTO CAGCTTCGC AACCTCTGAC GTCGGAGGCG CTTCCCGTTC ATCCGCCGC GAAGGGCAAG TAGGCTGGCG		AAAGGTGACT TTTCCACTGA GATCCAGTCG	COUTTAACCT GCCAATTCGA TOTTGTGCAG	ACCIECTAGA TOGAGOTICT TOTTECAGGA	CCTCACAGAT GGAGTGTCTA CTGGGAAGAA	Psil cocroccor crccaccoca	GCGATACGAG CGCTATCGTC CCACAGCTCG GGTGTCGAGC CCAGACGGGC
3301	3601	3701	3901	4101	4301	4501	4601

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									Am Change has		
4901	GOTOCOCTINO	AGGCTOGTCC	-	פטענאנאבועט			CICCACATAN	CTABACTCCA			
	CCACGCGAAC	TCCGACCAGG	ACCIACCACGA	בידוי היה	ניניראקאאוקני	מישרוירויראף י		200			
5001	Tecocoocor	GGCCCTTGGC	CCCCAGCTTG	CCCTTTCX:ALSO	אטייניהייריריה א	, נעזעטעטענעט	TOCAGACIPIT			GCGAGAAATA	
	AGGCGCCGCA	CCGGGAACCG	CCCGTCGAAC	CCCAACCTCC	TCCGCGGGGT	וא דויכוניכטדכ	ACCITCINAA	ACTCCCCCAT	CTCGAACCCG	CCCTCTTTAT	
					CACCETOTIC	CATTECACGA	GUCAGGARAG	CTCTGGCCGT	TCCGGGTCAA	ANACCAGGIT	
1016	GCTAAGGCC	CCFCATCCGT	-	TCCGGGGCGT	_				AGCCCCAGIT TT	TTTGGTCCA"	
					_				1/01118() 		
5201	Tecestal	TITTIGATEC	GIPTICITACC	TCTGGTTTTCC	ATTACTOCAGE	GICCACGCIC	GGTGACGAAA AOOCTGTCCG		TOTOCCCOTA TACACIACTT'S	TACACIACTTY	
	ACCOCCTACG	AANAACTACG	CAMGANTOG	AGACCAAAGG	TACTCGGGCA	CAGGTCCGAG	CCACTOCITY	TCCGACAGGC	ACAGGGCAT ATCTCTGAAG	ATCTCTCAA!:	
		Khol								-	
5101	AGAGGCCTVT	CCTCGAGCGG	TOTTECHEES	TECTECTEGE	ATAGAMACTC	GOACCACTCT	GAGACAAAGG	CTCGCGTCCA	CICCCACCACO	AAGGAGGCTA	
	TUTCOGNER	OGAGCTCGCC	-	ARGAGGAGCA	TATCTTTGAG	CCTGGTGAGA	CICIGITICS	GAGCGCAGGT	ccearcaige	THECHECGAT	
5.401	ACTORGACTO	GTAGGGGGGG		GGGGGTCCAC	Trachecage	METGARAGAC	ACATGTCGCC	CTCTTCGGCA	TCANGGAAGO	TOATTOSTIT	
	TCACCCTCCC	CATCOCCAGC	-	CCCCCAGGTG	AGCGAGGTCC	CACACITICITO	TOTACAGOOG	GAGAAGCCGT	AGTICCTTCC	ACTANCCANA	
5501	GTAGGITATAG	GCCACGTGAC	COGGRETICC	TGAAGGGGGG	CTATAAAACG	CONTRAGER	acentearce	TCACTCTCTT	CCGCATCGCT	GTCTGCGAGG	
	CATCCACATC	COGTOCACTO	-	ACTTCCCCCC	GATATITICC	CCCACCCCCG	CCCAACCAGG	ACTGAGAGAA	OCCUPACCOA	CAGACGCTCC	
5501	Charle Cartering	CARACTTARGETA	CICCTICTICA	AVAGCOCCCA	TCACTTCTGC	GCTAAGATTO	TCAGTTTCCA	MANACGAGGA	COATTICATA	TICACCAGO!	
	CONTRACAS	CCCCACTCAT		THEGECOGE		CGATTCTMC	AGTCANAGGT	THEFTIGUECT	CCTANACTAT	ANGTEGOACC'	
							Hireffil				
+01.0	e a constant	Cary-Hardy A. C.	PERSONAL PROPERTY.	The state of the s	ACAAAGACA	ATCITITION		CCTCCCAAAC	GACCCGTAGA	CACACCCTTYCA	
70.6	GOCCACTA	COGAAACTCC		CCTAGACCAG	retrireter	TAGAMMACA	ACAGITCGNA	CCACCGITTIG	CROOCCATCT	CCCCCAACCT	
					Pvul						
t na s		G CTANTOTARIO	GCAGGGTTTG	GEFFFFF	CGATCGCGC	GCTCCTTGGC	CGCGATGTT	ACCTOCACGT	ATTCCCCCCC	AACCCACCK.	
7000	GTCGTTGAAC	COCTACCTCG		CANABACAGO	CCTACCCCCC		GCGCTACAAA	TCGACGTGCA	TAAGCCCCC	THECOTOCCU	
5001	CATHYTANAA	AGACCOCTUCT.		GOCACCAGGT	σενεφειεσεν	ACCICCOCTTG	TECAGGGTGA	CANCOTICAAC	GCTOOTGGCT	ACCIPCTCCGF	
1	GTANGCCCTT	TETGCCACCA		CCGTGGTCCA	Cardiacoca	TCACCCCAAC	ACCICCCACT	GITCCAGTIG	CONCCACCOA	TOCACACC	
6001	GTAGGCGCTC	: GITGOTCCAG	CAGAGGCGC	COCCCTIVAGE	CGACCAGAAT	GGCGGTAGGG	GGTCTAGCTG	concreted	GCCCCCTCTG	CGTCCACCAT	
	CATCCGCGAG	S CANCCAGGTC	פתכתככככם	GCGGGNACAC	CCTCCTCTTA	כניניכניאורכיכ	CCAGATCGAC	GCAGAGCAGG	CCCCCAGAC	GCAGGGGT C.C.A	
6101	AAAGACCCCG	: COCAOCAGOC	OCCCOTCGAA	GTAGTCTATC	THECATECITY	GCANGTCTAG	ממכנותכות	CATCCCCGGG	COOCANGCGC	GCCCTCGTA1	
1	1111100000			CATCAGATAG	AACCTAGGAA	CCTTCAGATC	GCCACACCACG	GTACGCGCCC	OCCUMENCE	CCCCACCATA	
6201	CROTTICAGIO	3 GOOGACCCCA		TCCCTTAGCG	CGGACGTA	CATGCCGCAA	ATGTCGTAAA	CCTAGAGGGG	CICICIONGI	ATTCCAAGA"	
4	CCCAACTCAC	CCCCTOGGGT		ACCCACTCGC	GCCTCCGCAT	GTACGGCGTT	TACAGCATIT	GCATCTCCCC	GAGAGACTCA	TAAGGTTCTA	
6101	ATOTAGGGTA	A GCATCTTCCA	CCCCCCATGC	TOCCOCOCAC	GTANTCRTAT	AGTTCGTGCG	AGGGAGCGAG	GACCITCGGGA	CCGAGGITTGC	TACGGGGGG	
	TACATCCCAT	r cetagnager	ה פפנפננדאנפ	ACCGCCCCTG	CATTAGGATA	TCAAGCACGC	Tecentractic	CTCCAGCCCT	CCCTCCAACG	ATGCCCTICC	
6401	CHGCTCTGC	r COGAAGACTA	1 rendection	GATEGCATET	GAGTÍNGATO	ATATOGITING	ACCCTCCTCCAAG	ACCITICAAGC	TGGCGTCTGT	DAGACCTACK:	
))	GACGAGACGA	A GCCTTCTGAT	r AGACGGACTT	CTACCCTACA	CTCAACCTAC	TATACCAACC	TOCOACCTTC	TGCAACTTCG	ACCCCAGACA -	CTCTGGATG	

Figure 150

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6501	GCGTCACGCA	COANGGAGGC (GTACKIAGTCG	CGCAGCTTGT	TCACCAGCTC ACTCATACTAG	GGCGGTGACC TGCACGTCTA			GTCCAGGGTT	TCCTTGATGA AGGAACTACT
6601			TETTTTTCC		CAACTCCTVT	ANTITITIONS TITIES	CCAGAMAGGT	GTACTCTTOG CATGAGG	ATCGGAAACC TAGCCTTTGG	CCTCCCCCT* :
6701			TGTAGAACTG	GTTGACGGCC	TGC:TAGGGGG AGC:ATCCGCTG	ACKTARGGGAA Textitagggaa	TTCTACCCCT	AGCGCGTATG TCGCGCATAC	CCTOCCCCCC	CTTCCGGAC
6801	GAGGTGTGGG	TGAGCGCAAA	GOTOTCCCTG	ACCATGACTT	TGACKITACTVS ACTCCATVIAC	GTATTTGAAG	TCAGTGTCGT	COCATOCOCC	CTGCTCCCAG	AGCAAAAAGT TCGTTTFTTCA
6901	CCOTCCCCTT	AAACCTTOCO	GGATTTGGCA	CCCCCTTCCA	CTGTAGGAAC	AACAGTATCT	TTY COCCOCC AAGGGGGGGG	NGGCATAAAG TCCGTATTTC	TTGCGTGTGA	TOCGGANGGT. ACGCCTTCCT
7001	TCCCGGCACC AGGCCCTGG	TCGGAACGGT	TOTTAATTAC	CTRICACCARA	AGCACGATCT TCGTGCTAGA	CCTCAAACCC	CENCENCETTS CANCENCANC	TOCCCCACAA ACCGGGTGTT	TOTAAAGITIC	CANGAAGCGC OFFCFFYCGCC
7101	GGGATGCCCT	TONTOGNAGO ACTACCTTCC	CAATTTTTA	AGTICCTCGT TCAAGGAGCA	AGGTGAGCTC TCCACTCGAG	TTCARROCCAG AAGTCCCCTC	CTCAGCCCGT	GCTCTGAAAG	GOCCCAGTCT	GCAAGATGAG COTTCTACTC
7201	GOTTOGAAGC	CHCCATICAC	CTCCACAGGT	CACHGGGGATA	TAGCATTIGE ATCGTAAAGG	ACCACCACCC	GNANGGIECCT	ANACTOGCGA	CCTATGGCCA	TTTTTTCTGG
7301	CCACTACGTC	TAGAAGGTAA	OCCIONATO TO COCCIONATO COCIONATO COCCIONATO	TTCCCAGCGG AAGGGTCGCC	TCCCATCCAA AGGGTAGGTT	CCANOCCCC	TAGGICIOGO	GCGCCGTCAGT	CTAGAGGCTC	ATCTCCGCCG
7401		CCACCATGAA GOTCOTACTT Pvul	CCCCHCCHCC	GOGCAGGAGC TRCTTCCCAA CCCGTGCTCG ACGAAGGGTT	AGGCCCCCAF TCCGCGGGTA	CCAAGTATAG GGTTCATATC	GTCTCTACAT	CCTANGTGAC	AAAGAGACGC TTTCTCTGCG	AGCCACGCT
7501	GATOCOAOCC CTACGCTCOO	GATCGGGAAG CTAGCCCTTC	AACTGGATCT	AACTGGATCT CCCGCCACCA TYGACCTAGA GGGCGGTTGT	ATTGGNAGAG TANCCTCCTC	TOGCTATTGA ACCGATAACT	TCTCCTCAAA		CTGCGACGGG	CCGAACACTC
7601	CACGACCGAA	CACGACCGAA AACATTITIG		OTOCOCAGTA CTGGCAGGGG CACGGGTCAT GACCGTCGCC	TCCACCACCT ACGTCCCCCA	GTACATCCTG CATGTAGGAC	CACGAGGITIG GTCCTCCAAC	ACCTGACGAC CGCGCACAAG TGGACTGCTG GCGCGTGTTC	CGCGCACAAO GCGCGTGTTC	CITICGICITCA
1011	CCCTTAAACT	OCCCCTCGCC COOGGAGCGG	TCGCCCCAAA	GGCTGGTGGT	CTTCTACTTC	GGCTGCTTGT CCGACGAACA	כנבדלמארכפ ד המיאתכדעיהה	CTGGCTGCTC	GACCCCTCAA	ACCCACCTA:
7801	CCTOOTOOTO			AGATGTCCGC TCTACAGGCG	GCGCCCGCCA	CCCACCTTCA		TENCIACATE GEGENGATEG GAGETGTECEA ACTIGITIGIAG EGEGICITACE CINCACACACT	CAGCIGICCA	TOGTCTCGAG ACCAGACCTC
7901	CTCCCGCGGC	GTCAGGTCAG	GCGGGAGCTC	CTGCAGGTTT GACGTCCAAA	ACCTCGCATA TOGAGCGTAT		GACOGIGTCAG GOCGCGAGGT CTGCCCAGTC CCGCGCCCGA Kanl	AGATCCAGGT	GATACCTAAT CTATGGATTA	TYCCAMGGGC. AAGGTCCCCG
8001	TEGITEGICS	RESTRUCTES COCCOTCGAT ACCAACCACC GCCGCAGCTA	COCATOCANG	AGGCGCATC TCCGGCGTAG	CCCASCARICAC	GACTACCCAT CTGATCCCAT	מונים בים בים בים בים בים בים בים בים בים ב	GACTANGONA CHERCACO GOCGOTAGOC COCCOCOTA TECTTAGATA	COCCOCCCCAC	TCCTTGGAIY; AGGNAGCTAL

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GCGCGCGTTC CCCCGCCCCT: CCGCGCCCCT: CCGCGCCCACT: CCTCGTTATCCC: GAACTATCC:	GCCGTACTO: CCGGTACTO: ACATTOAGC!	TAACCCAGC: ATTGGGTC(X)	CGCCCGCTC	TCANTCTCC: AGTTAGAGGA			AGCGAGTC(** TCGCTCAGG	GGCGGTTCGCK: CCGCCAGCCC	TCCGGCCTGC AGGCCGGACG			ATCCATGTCC TAGGTACAGG
COTCGRICOCO GRAGACGRICO GARGACGRICO CTTCTGCTCC CCTCGRICTTGT GGACTCARCA	GCGAGGTCCT TOGAAATGCC CGCTCCAGCA ACCTTTACGC CGCGCATGAC CACCTGCGCG GCGCTACTG GTGGACGCGC	AAGAAGTACA TTCTTCATGT					GAGGGACCTG	CONSCIONA DECAROCADO CONTROLIXA GENECACION CONTROLIXA CONTROLICA C	TOTCCTTOGG ACAGGAACCC			ACTURANGTO TGACCTTCAG
		TTCTGCCACG			-		CTCCGCCGC	CGTGGCGGGC			CCCTCTTCCT	CACTCCCATC
	CACOGTAGEG GTGCCACCGC GCATCGCAGG	TGGCTGTGTG ACCGCCACAC		CACCTCCCC TCAAAGGCTA GTGGAGCGCG AGTTTCCGAT		GCCCAGTTCGG	TCTCTAGGTA ACACATCCAT	GGCTGAGCAC CCGACTCGTG Sall	CTACCAGCTO		: מדאהמדשמכה ז כאדככארטפכ	ATATACCEGAC GACGTGGACG
CCHRACCCRC GATCANCTCC CAACTAGAGG GAGGCTGGC CACCCRACCG	CCACTACACA GCCCCCTTCC CCCCCTTCC	THEACCCITES	CCTCCTACAA		GGGACACGGC	TrTCGCGGGG AGAGCGCCCC	CAACAATTGT GTTGTTAAGA	TCGCAAGGTA AGCGTTCCAT			CTCAAACUGG	ATATOGCCTO TATACCGGAC
NGBSSSSCT TUCKT CACCA CURACICAGE CATATIGAGE CAGANACIEG	CTCCTCGACA TCTCCCGGAGGGGGGGGGGGGGGGGGGGG	NANGAGGTAG	DECETEANGE COCYCEANTAG CENTOTAGAAA COCGGAGTICC GCGAGGIACC GCAGGAACT	AGCTEGACGA CAGTRITEGEG TEGAGCCGCT GTCACAGCGC	TGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	GCGCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	CGATCCATCT GCTACGTAGA	CCAGTCACAG			מתריטילים	CCCTCCCCTA
CCCCGANGE NGG GGGGCCCA TCC GCGANCGGGA TGA GGCTTGGGT GGG GANTINGGGT GTG GTTAAAGGGA CAG	CTCCTVGAGA GAGAGCTCT CAGACGCGGC GTCTGCGCCG	GCACGCGCTG			CTOCCOCCGG GACCGCGCC	CTCGGTGACG	ACGGCGCTAA	AGGCGTCTAA TCCGCAGATT		GCTTCGTTTT CGMGCANAA	CTATCGCTGC	GCGCTGTTGC
	COATCTCTTK GCTAGAGAAA TCCCTKGTTC AGGGAGCAAG	G GCGTAGTTTC C CGCATCAAAG EGOHV	GARTECTICA TATECECECA CTANGEARCT ATAGGGGGTT	AAGACGGATG TTCTGCCTAC	GOCCICCCT TCTTCTTCTTCTCTCTCTCTCTCTCTCTCTCT	CCGCGGGG GGCGCATGGT GGCGCCGCTG CCGCGTACCA	GOCTOCCATO COCCAGGAT CCGACGOTAC GCCGTCCCTA Xhol	CTCTCGAGAAAA	TGCTGATGAT	CATACCCCCAG	TCTCTTGCAT AGAGAACGTA	
MOCCOTCAC GCCACCATA TTCGCCACTO -COCCACTACT GCTGCGCGC TACGTTCCTC CAACGCGCGC ATCCAACAAC GAAAGAGAT TCGACACAAAT CTTTCTCTCA AGCTGTCTTA	ATGAACTOCT TACTTGACGA CGTTGAGGCC	OCCGNAGACO CCCCTTCTGC	GATTCGTTGA	CCTCCTCCAG GGAGGAGGTC	CCGGAGGGGA	CCCCCCCCTC	GCCTCCCATG	ATCCGAAAAC	GCGGAGGTGC CGCCTCCACG	OCCOCCACCO	TTGTCCTGCA	00
NTGCATCTAN TACGTAGNTT AGGACCTTCCTCCACCA GCTTGAACCT CGAACCTTGGA	GATCTCGGCC CTAGAGCCGG TGCGAGAAGG		TCGC/ACGTG AGCGTTGCAC	ACCONTANCT TOCCAATICA	CPTCCATAAG	GATCATCTCC	GTTOGCOOOO CAACCOCCC	CATCGACCGG	GINGTITICTO CAACAAAGAC	TOAATGCGCA ACTTACGCGT	CICCITICCITC	GCCCCTCATC
8101 8201 8301	8501	8601	8701	8801	8901	9001	9101	9201	9301	9401	9501	9601

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						CTITCGCTTT	TOCCOCOMIC	GCCACGACGC	CCCCTGTAGG	GCCGATCCTC	TCCCTCTCCT	ACTEGRACIA COCOUTIONS Handin	AAAGTTT		COCCCICCCG
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Figure 15I

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Figure 15J

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Figure 15K

Eorav TCGGCACCAG	CAATATGAGC	GGTGGCGCT	TCAGCTGGGG	בירימרותיואים האיניתיומיים	ACCRICATTA . TYGCCGTAAT	AAAATTTYYGG TYTYAAAGCC	TTCCACCGTT AAGGTOGCAA	ANGANCTATO TTCTTGATAC	GCAGCAAGG	
CTOGAACAGC GACCTTOTCG	CTOGRACIOC CITATACIOS CTOGRACIOC ACCACAGGICC GACCITOTOG TCOTGACCCG	AGATGCTGAG TCTACGACTC	GGATAAGTTO				GATGGCCTGG CTACCGGACC	CCTCTGGCAT	TAGCCCCCA!	
GTGGACCTGG	OTOGACCTOG CCAACCAGGC	AGTGCAAAAT, TCACGTTTTTA	ANGATTAACA	GTANATITGA CATTETAACT	TOCCGGCCT ACCIDENTAL	CCCOTTAGAGG FASTCATCTCC	AGCCTCCACC TCGGAGGTGG	GOCCOTOGAG CCGGCACCTC	ACAGTGTCP: TOTCACAG/*!	
CAGAGGGGG		CGFCCGCACC	CCGACAGGGA	AGAMACTETS TETTTEAGAG	CACTGCGTIT	TAYACGAGCC ATCTGCTCGG	TCCCTCGTAC AGGGAGCATG	GAGGAGGCAC	TAMAGCAM": ATTTCGTTC	
	< ⊢		TCGCCCCAT GGCTACCGGA AGCGCGGGTA CCGATGGCT	CACCIACCCCG	AGCACACACAC TCGTGTGTGTGG	COTAACGCTG	GACCTGCCTC	CCCCCCCCA	CACCCAGCA:	
	AAACCTOTOC TOCCAGGCCC	GACCGCCGTT	GTTGTAACCC	GTCCTAGCCG	CCCCHCCCTG	20020202020	CCAGCIGOTCC GOTCGCCAGG	OCCINGCACC	COCCCOTAG	
	0 6	ACACTGAACA	CGTAGCACCC	TCTGGGGGTG	CNATCCCTMA	AGCGCCGACG TCGCGGCTGC	ATCCTTCTGA TACGAAGACT	TAGCTAACGT	GICCITATIONS CACICATACA	
		_	CCTCGACGAC	AGGGGGGGG	CCCCCCCTTT	CCANGATOGC	TACCCCTTCO ATGGGGAAGC	ATGATGCCGC TACTACGGCG	ACTOCITOTA TEMENDA: "	
		ACCCTCGGA TGCGGAGCCT	GTACCTGAGC	CCCCCGCTCG	TOCAMPTERIC	CCGCGCCACC	GAGACGTACT	TCAGCCTGAA AGTCGGACTT	TANCAAGTIT	
	00		GTCACCACAG	ACCRETCECA	הכהדדדהאה הפכאאניזהנ	CTRCGGTTTCA GACGCCAAGT	TCCCTGTGGA	CCOTOAGAT	ACTCCGTACT TCACCCATGA	
	00	CTAGCTNTGG	CACTATACC	TGTGCTGGAC ACACGACCTG	ATGGCTTCGA TACCGAAGGT	CCTACTTTCA	CATCCCCCCCC	GTGCTGGACA	OCCCCCCTA CCCCCCCCATA	
			CCCCCTCACT	CCCANGRATIG	CCCCAAATCC	TTGCGAATGG AACGCTTACC	GATGAAGCTG	CTACTCCTCT	TGAANTAAN ACTITATTIK:	
	CTAGAAGAAG AGGACGATGA	CAACGAAGAC	GANGTACACG	AGCAAGCTGA TCGTTCGACT	GENGEANANA	ACTEACRTAT TRACTECATA	TTGGCCAGGC AACCCGTCCG	CCCTTATTCT	GCTATAAATA CCATATTTAT	
				ACCTANATAT TGGATTTATA	GCCGATAAAA	CATTICAACC	TGAACCTCAA ACTTGGAGTT	ATACGAGNAT TATECTETTA	CTCAGTGGTA	
	•	CAGCTGGGAG	AGTCCTAAAA TCAGGATTTT		CAATGAAACC	ATH:TFACOGT TACAATGCCA	TCATATGCAA AGTATACGTT			
	GCCCAAGCCA TICTTGTAAA CCCCGTTCCGT AAGAACATTT	GCMCMMT	GGAAAAGCTAG CCTTTCKATC	AAAGTCAAGT	CCTTTACCTT				TTACCACTA!	
	ACTTGACTCC TAAAGTGGTA TGAACTGAGG ATTTCACCAT	TTGTACAGTO	ANGATGTAGA	TATAGAAAGG ATATGTTTGG	CCAGACACTC	ATATTTCTTA	ATATTTTTTA CATGCCCACT TATAMGAAT GTACGGGTGA	ATTANGGANG TAATTCCTTC	CATTGASTOI:	

Figure 15L

SCAC COCTIANTAGE COTO CCCATTATAC TOAT TCCATTGGTG ACTA ACGTAACGAC GATO AACTTCCTTC ACTA AACTTCCTAC			CTUG CCTUGGGTMC GACC GAACGCGATA CCTG CCGGGCTCAT GAAC GGCCGAGTA		MOTE CTTTNACO! : FTCAG GANTTGC! : FTTE CGCGCCTGC? SNAAG GCGCCACTC.	SCAND ANGGARCTHY SCANC TACCITICALA ACCA CITTINAANTY FIGER CANCITITIAA		GACAGGCCTA CCCTGCTANI' CTGTCCGGAT GGACGATTG CATCCCATTC TCCNGTANGT GTAGGGTNAG AGTTCATTGA
TT ACANCAGCAC AA TOTTOTCGTO CCT PTTCCTTGAT GA AAACGAACTA CA ACTGAAGATO		-	NCC GCAATGCTGG TOG COTTACGACC CCT CCTTCTCCTG 3GA GGAAGAGGAC		CCA ACGACCAGIC GGT TGCTGTTCAG CTG GGCGGCTTTC GAC CCGCGGAAG	TAT CCCTACCTAG TAT GGGATGGATC TTA CCCCCACGA AAT GGGGTTGCT	AGC TANCTATANC TCG ATTIGATATTG CTG GTGTATATTG CAC CACCTACTAT	ATCCOCOTTC CTOTCCGGAT TACCCCCTTC CTOTCCGGAT CCCTTTGGCG CATCCCATTC GCGAAACCCC GTACGCTAAG
			T AACCACCACC A TTGGTGGTGG A TTAAAAACCT T AATTTTTGGA		T TTGCTGTGGT T CCCGCAACTG T CCCGCAACTG T CCCGCAACTG		NC ANNIGCTAGE NG TITACGATCG NG CCGTCAGGTG NG CCGTCAGGTG	
TTTTATTGGT AAAATAACCA ACAGAGCTTT TGTCTCGAAA	CTTANTANCE AACAGGTCAG TTGTCCAGTC	CCCAACCITYT CCGTTCCACA ACACCTACGA TGTCGATCCT	CAACCCATTT GITGGGTAAA TTCTTTGCCA AAGAAACGGT		CATGCTTAGA GTACGAATCT TCCATCCCCT	_	AAGGACCATG AGCCCATGAG TCGCGTACTC	TOCCCCACC ACCCCATGG Poul Poul TYCCATCCTA ACCCTACCTA
TTAGGGACTAA AATCCCTGTT AGACAGAAAG TCTGTCTTTG CCAGATGTTA	GCTCTACAAT TAAAACCTAA ATTTTGGATT	CANTCTAAAT GTTAGATTTA GATAACCCAA CTATTAGGGTT	TYGACAACGT ACCTGTTGCA GCCTCAGAAG		CGCTTGAGGC GCGAACTCCG CGTGCCCATA		GTTTCTGACC AGAMCTTCC TCTTTGAGG	TTGGCTACT AACCGATGGA AAAGTTTCTT TTTCAAAGAA
TACAPTGCTT ATGTAACGAA TAGATTTGCA ATCTAAACGT CAGCTATGAT	GTTACCANGG GAATGGTTCC	CCATCCTTTA GGTACCTTTA AAAAATTTCT TTTTTAAAGA	CTTGACTATA GAACTGATAT ACATCCAGGT	Pstl TCTTFCAGAGC AGACCTCTCG	ACCOCCTCCA TCX CCGAGGT ACGCTAGGAA			TCTGGATTTS AGACCTAAAC TTACCCAGAA ATGGGTCTT
CAGGCCTAAT GTCCCGANTA AATGCTGTTG TTACGACAAC	TCCGACAACT TACAGAGACT ATGTCTCTGA	AATAATTTIG TTATTAAAAC CTTCCAACGT GAAGGTTGCA	ACGCTCATCC TGCGACCAGG GTGCCCTTCC	TTAACATGGT	GGCCCACAAC CCCGGCTGTTG ATACCCGCCA	CATCACTGGG GTAGTGACCC CATTAGCTTT GTAATGGAAA		
CTATGCCCAA GATACGCCTT ATCGCAGTTG TAGCGTCAAC	TACACCTTAG GTGTGATTAA CACACTAATT	AAGAGTTGGA TTCTCAACCT AAGTACAGTC TTCATGTCAG	ACCTTGGAGC TGGAACCTCG TGGTCGCTAP	ACCANGGATG TCCTTCCTAC	AGAAGGGTA GCTCTACCCT	COMPANICOS ANGRANCEC TECETETOGG AGANGGETGGC TECETETOGG	GGGTTACAAC CCCAATGITIG AGCTACAAGG	
GOCCAACAT COGGITGITA COGGCOATIC GCCCGGITC GTACTITIC GTAC	CATGAAAAGA CCACTGAGAG GGTGACCCTC	AAAATGAAAT TTTTACTTTA CGACAAGCTA GCTOTTCGAT	TOCTACATTA ACQATOTAAT TOCTGGGCAA	OTCOALCEUTT OTCOAACTTC CACCTTGAAG	TACCCACCT ATCCGGTGGA CCGCCAACAT	CCTTAAGACT CCTTAAGACT CCACACCTTTA	TTGACGGGA AACTGCCCCT TATCCCAGAG	
AGANCTANTO O TICTTGATTAC O GOTOTTCTOO CCACANGACO ATAGANCO	TATCTTGGTC C TTACTGCTTT C AATGACGAAA C	AAAAGTCTAT TOTATTTGCC TOTATTTGCC TOTATTTGCC	U 0 E	ACACCTACGA TOTAGONTOCT	CATTIGGGTT GTAAACGGAA TATCTCTCCG	ATAGAGAGGC CCTTCACGCG GGAAGTGCGC TTACCTCAAC	AAGCGCTCAG TTCGCGAGTC AGGGCTTCTA	OCCEDATOCET TECCCETATC AGGGGATAG
19301	1961	19701	19901	20101	20201	20401	20601	20801

Figure ISM

Bartill **********************************		ingin .		GOCCIGATORS C	COACTCAAGC	AAAGCGTAC . TTTCGCATGT	CACCATGAN' GTGGTACTT '	OAGCGCCAC" CTCGCGGTR	CTTTCAATAA GAAAGTTAT''	CGCATCGCTA	TCACTCCAC/. AGTGAGGTG!	GATACACAGS CTATATGTCY	GTTGCTCAGG	TGACCGTCCC ACTCCCACGG	CGCAAGACTT	ACCACATTIC GGCCCCACCG GFTCTTCACG TGGTGTAAAG CCGCGGTGGC CIAGAAGTGC
		ALL KALGE		TAGTCAATAC ATCAGTTATG	TTCTGACCAG AAGACTGGTC	AAGTCCACCC TTCAGGTGGG	ATCACAACCC TAGTGTTOOG	CAGCITICCIO	ACTAGACACA TOATCICTOT	OCTIVE TOCCO CCANGACOCC	OCTUBARGITT CCACTTCAAA	COCOAOTTOC GCGCTCAÁCO	GGTCCTCCGC	CATCAAAAGG GTAGTTTTCC	AAGAACATGC	GGCCCACCG
CATGACTTIT	ATCGAAACCG	TACTITION	CONCOTTOAC	GCCTGCGCCA	CCTTTOGCTT	AACGCTCGAA TTGCGACCTT	ACTCCCATGG TGAGGGTACC	AACAGCTCTA	AAAATAATGT	AAATCAAAGG	GCGGCAGCTC	GCCCTGCGCG	TCCGCGTCCA AGGCGCAGGT	ACCOPAGEO TOCCATCACC	GCCTTCAGAG CGGAAG I: TC	ACCACATITIC TOGICITAAAG
ACGCGCTAGA	COCCOSTOTO	מוכניברמניעו	CGAGGTCACT	ACACAMGCTC TGTGTTCGAG	CTCTTTCAGC	ACCGCTGTAT TGGCGACATA	CTOCCCCCAA	COCAACCAGO	TOTCACTTGA ANAACATGTA ACAGTGAACT TTTTGTACAT	COCCOTITINA	AAACTCAGGC ACAACCATCC TTTGAGTCCG TGTTGGTAGG	TGGGGCCTCC ACCCCGGAGG	GGAGATCAGA	TTGCACTCGC AACGTGAGCG	GAGCCTTTGC CTCGGAAACG IIGH	CCTCTAGACG
AACTECCECC	ACCACTCCCA	Testroscer	COGCOGIACC	THEFTETCEC	AACATGCTAC	TCTTCCCCCG	CCTTTCCCAA		TGTCACTTGA ACAGTGAACT	TTGCCGTCTG AACGGCAGAC	AAACTCAGGC TTTGAGTCCG	AAGTCGCAGT TTCAGGGTCA	COCTUTTOTO	AGGCTTTGAG TCCGAAACTC	AAAGCCACCT TITICGGTGGA	CCTCCCTCTT
ACAGACCTAG GCCAAAACCT TCTCTACGTC AACTCCCCC ACGCGCTAGA CATGACTTTT	AGAGATGA TA	באממניאכאניז - -	TTGTTGTGT	TTTCCAGGCT	CGCACTGAAA	CGCCATTCCT	TTTCTCCACG	AGGTACAGCC TCCATCTCGG	CACTRCTTT	ACCCCCACCC TOCCTOTOGG	TGCTCCACTT ACTAGGTGAA EcoffV	CCATATCTTG	Charachara	מכטכעלטט נופכטכעלטטפ	GATCTGCTTA	TRIATTROCCO OACAGICCOCO OACGITICACO CAGCACÓTTO COTCAGITAT GOAGAITATAC ACCACATATAC GOCCCCACCO GITICATICACO ACTAACCOGCO CAGCACOTGO CAGCATAGAAC CAGCACATA COTCATAGACO TOGITIAAAO COGCOGAGO CAGAAAATAC
RCCANAACCT	CITTGACATG GTCCGTGTGC	GAMACTICENC CAGGEORICS	TTCGTTCTAG	TEACAAGCGC ACTGTTCGCG	CCCACCTTCC	TCCGCCGTAG ACGCGGCATC	CTCCTCCATG	ANCAGECCC AGGENCAGCC	TTAGGAGCGC AATCCTCGCG	GTGATTATTT	TOGTGTTTAG ACCACAAATC	GOTCOCCCC	GTCOTCACG	CCCAAAAAGG	TAMAGCCTT	GTCGTTCACG CAGCACGTGC
ACAGACCTAG			TATTICITED	TCCCCTCCTA	CTACCGGAAA	GAGTCACTCC	GIGGACTATT	CTCCATGCTC	AGTOCGCAGA	ACACTCTCGG TGTGAGAGCC	GTTGCGATAC	GCGTTTAGCA CGCAAATCGT	TCAGCGCCCGG AGTCGCGGCC	TAGCTGCCTT	AGCCCCTCCA TCCCCCTACCT Still	GACAGGCGC
			ACCCACACAC TOCOGRETTO	CCATATFITT	COCATOTORC	CAAACTCATG				TITITATITIGE AAAATAAACA	OCAGGGACAC COTCCCTGTG	CATCACCAAC	TOGANCACTA ACCTTGTGAT	1CAACTITIOG AGTITGAAACC	CTTAGGATAC CAATCCTATG	TGATTGGCCG ACTAACCGGC
			AGCCGGCCGT	TOOPTIOTOGO ACCAACACCC			CCCCOORTIO					GCTGCGCAC	GATGCAGCAC	OCGAACOGAG COCTTOCCTC	COORCIOCOC	GCCGGNAAAC CGGCCTTTTG
21001	21101		10212	21301	21401	21501	21601	21701	21801	21901	22001	22101	22201	22301	22401	22501

Figure ISN

ATGCTTCCGT TACGNAGGCA	CNANCONCTE OTTRICTEIN*	CCAGCICTT-1 GSTCCAGAC CGCGCAGCCT	GCGCOTCGT 1 CCTCTTGCGT GCAGAACGCA	GCTGAAACCC CGACTTTC#3G	AAGAAAAAA	OCARRIAGECT ACCTERCIC	TEAGTECOM:A ACTEAGETET	OTCOMBETAL COCCOCATION CAGCIFICATION ANAGEMEN CONGRESON' TITINGSTICE GOTCOTOTION	7. S	CACCOCOCT GREGOCOCOCA CTATCACATT GATACTICTAS	מפאר
ATTTATCATA I				CCOGTCGGTT	TCTTCCCGCG	CTCAGAAGGA	ACCAACCCCC GATCATGGAG CTAGTACCTC	GICCARGCAC CAGCTCCCTTO AAAAGCAAGA TTTTTCGTTCT		CACCTATICT GTGGATAAGA TGCTTGCCAC ACGAACOOTO	CGCTGTCATA GCGACAGTAT
	ATCCTTCTAG GTCACCTCTG TACGAACATC CAGTGGAGAC	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC			GCCCGAACCC	CAGAACACTA		CACCTTCCCC GTGGAAGGGG ACAGAGGATA TGTCTCCTAT		CTACGAACGC GATGCTTGCG GTGCCAGAGG CACGGTCTCC	TOCOGCAGGG
GEGEGERICE COTTINGET COTCACE ATTICANTO COTCETT COCCECT COCCECT COCCECTOR GENERAL CONTINUE TANGETAGE GENERAL		CAGCTGCAAC GTCGACGTTG TTATCCACGT	AATAGGTGCA CACTITICCGC GTGAAAGGCG					ACCCCCTAC TCCCCCATC CTCACTACCA GAGTCATGGT	GTOGGAGACO	TCAGCCTTAGE AGTCGGAACG CGTATTAGC	ARCGGACANG CAQCTOOCCT TQCKGCAGGG CGCTGTCATA TCGCCTGTTC GTCGACAGGA ACGCCGTCCC GCGACAGTAT
CCACTGTAGG	COCAGOGO CAPOTACACA COCAGOCO TOCACACA GOODICA COCAGOSACACACACACACACACACACACACACACACACACACAC	TEGITGANGET ACCACTTCEA CTTTAGATEG	GAAATCTAGC	GCTTACCTCC		CCACACGGGG CCACACGGGG	CACTICCCCT GACCATITICC CCOGITAAAGG	GAAGGCGGGA CTACGGCGGT ACGAGGAGCG	CTACCTAGAT	TATCGCCTAC ACTTCTACCC ACTTCTACCC TSAAGATGGG	AGCGGACAAG
CCTPTRACT GCAAAAGCGA	נאטכנאטאט	COTCACANG CICTICITAC GCACIOTITC CACAACAACG	GACHTESTICA GECASTASTI INJONETICASE GAGATTENAGE CONTACTOR ACTIVIANGE GAGATTENTE	CGCACTETEC CCGTSACACG		CCGCGGGCTC GGCGCCCGAC GGAGGCGCGGCG		TEGECACCAC CONTYCCACC AGCGGTGGTG GCGANGFRG AGGTTTTGTA AGCGANGACG TCCAAAACAT TCGCTTCTGC	GOCATOGICA		ACTOCANGAT ACCCCTATCC TOCKGTGCCA ACCGCAGGG TGACGTTCTA TOGGGATAGG ACGGCACGTT TGGCGTCGG
CGCGCGATACC	CCCACCCCAC	CCTCACAAAG GTCTTRTTRGC GCAGTGTTTC CAGAACAACG	CCGTCATCAA	ATTCAGCCGC TAAGTCGGCG	-	ACCTUCATOR TECAGETACE GOOGECERS					ACTOCANGNT ACCCCTATCC TGCCGTGCCA TGACGTTCTA TGGGGATAGG ACGGCACGTT
כדונינידונאמכ האוויאמהוכה	TCGNTTTT.AG	GCCCCATCAT CGCCCATAGTA	GACTICACTCA GTGAACCAGT Fvd GACACGATCG	CCAGCAGAAG	-	TCCOCCGCCC AGGCGGCGCC		CCCTCTGAGT GGGAGACTCA AGCAGGACCC			F ACCCCTATCG
TOCTAGACTG	CHCGCCT	Psil TOCAGGAATC ACGTCCTTAG	CCAGACTTC GGTCTCGAAG CTCCCACGCA	COCCCACTO CCCCCCACTO CCCCCCCTCACTO	GCGCCACATC	ANTOGCCAAA TTACCCGGTTT	CCCGAOTAGG CCCGCTCGGG GCCCGAGCCC		ACGAGGAACA		
ATCTTGGCCT TGCTAGACTG CTCCTTCAGC TAGAACCGGA ACGATCTGAC GAGAAAGTCG	GTAGACACTT	CAGGTACGCC GTCCATGCGG	CATACOGCO GTATGCCOGC CCATGCCCTT	GCGCATACCA CCGCATACCA GCCGTATGGT	ACCATTIGIA	TCTTGGGCGC AGAACCCGCG	GACCOCOTC CCACCOCOTC	AGAAGGACAG TCTTCCTGTC GGAGGAGGAA	GCAGAGCAA	GCCCCTTAT CCCCCCTAATA ACCCCCCAAA	TTTTTCCAAA AAAAAGGTTT
22601	22701		22901	23101	23201	23301	23501	23601	23801	23901	24101

Figure 150

PMRKAdSgag MERGB2

24201	CCTCGCTCAA		COANGTRECA ANALTETITES GETTEAGGGT TITTAGAAAC	AGGGTCTTTG TCCCAGAAGC	ACGCCCACGAC TGCCCTGCTC	ANGICKTORTEG TTYTGCKTORTEG	CAAACCICTET G†TTGCGAGA	OCANCAGGAA CCTTGTCCTT	AACAGCGAAA	ATGAAAGTEA PACT'FTCAGT
24301	CTCTGGAGTG		TTGTTGGAG TCGAGGTGA AACCACCTTG AGCTCCACT	CAACGCCCCC	CTAGCCCTAC	TAMANCICAG	CATCGAGGTC	ACCCACTITO	CCTACCCGGC	ACTTAACCTA
24401	CCCCCCAAGG		AGTCATGAGT TCAGTACTCA	GAGCTGATCG	TREGREGATE	GCAGCCCCTG	CACAGGGGATO	CAAATTTGCA	AGAACAAACA TCTTIGTTTTGT	GAGGAAGGG""
24501	TACCCGCAGT			GCTGGCTTCA	AACGEGEBAG TTGCGCGCTC	CCTGCCGACT	TGGAGGAGCG ACCTCCTCGC	ACGCAAACTA TGCGTTTGAT	ATGATGGCCG TACTACCGGC	CAGTY:CTCGT GTCACGAGGA
24601	TACCGTGGAG ATGGCACCTC		CTTGAGTGCA TGCAGCGGTT GAACTCACGT ACGTCGCCAA Byll	CTTTGCTNAC	CCGGAGATGC	AGCGCAAGCT	AGACTAAACA TCTCCTTTGT	TTGCACTACA	CCTTTCGACA	GOOCTACGTA
24701	COCCAOGCCT	88	CAACOTOGAO OTTGCACCTC	CTCTGCAACC	TGGTCTCCTA	CCTTGGAATT GGAACCTTAA	TTGCACGAAA	ACCOCCTTOG TOGCOGAACC	GCAAAACGTO	CFFCAFFCCA
24801	CGCTCAAOOG		CONGOCOCOC COCONCTACO OCTCCOCOCO GCGCTGATGC PSII	TCCGCGACTG. AGGCGCTGAC	CCTTTACTTA	TTTCTATGCT AAAGATACGA	ACACCTGGCA TGTXX3ACCGT	GACOGCCATO	OGCOTITION CCGCAAACCO	AGCAGTGCT+' TCGTCACGAA
24901	CCTCCTCACO	AACCTCAAGG	AGETGCAGAA	ACTGCTAMAG TGACGATTTC	CNAAACTTGA GTTTTGAACT	AGGACCTATG TCCTCGATAC	CTOCCGGAAG	ACCAGCCT	CCOTOGCCCC	OCACCTOSCY: COTOGRACCOC:
25001	GACATCATTT	TCCCCCAACG AGGGGCTTGC	CCTGCTTAAA	ACCCTTCCAAC TCCCACGTTC	ARKSTCTOCC TCCCAGACGG	AGACTTICACC TCTGAAGTGG	AGTCANAGCA TCAGTTTTCGT	TOTTGCAGAA	CTTTAGGAAC	TITATCCTA".
25101	AGCGCTCAGG TCGCGAGTCC	Antethoccc Tragaacgog	GCCACCTGCT CGGTGGACGA	GTGCACTTCC CACGTGAAGG	TARCCIACTIT	GTISCCCATTA	AGTACCCCGA TCATGGCGCT	ATCCCCTCCG TACGCGAGGC	CCGCTTTGGG	GCCACTGCTA COGTGACGAT
25201	CCTTCTGCAG	CTAGCCAACT	ACCITIOCCTA TGGAACGGAT	CCACTCTGAC	ataatccaag tattacctec	ACGTVARCOG TYACOGTCTA TYCACTCCCC ACTCCCAGAT		CTGGAGTGTC GACCTCACAG	ACTOTOGOTO TGACAGCGAC	CAACCTATGG
25301	ACCCCGCACC	OCTCCCTOOT CGAGGGACCA	TTGCAATTCG	CAGCTGCTTA	ACCAMAGICA TGCTTTCAGT	AATTATCGGTT TTAATAGCCA			CTCGCCTGAC	GAMMAGICCH CTTTTCAGGT
25401	CGGCTCCGGG	GTTUANACTC	ACTCCGGGGC TGAGGCCCCG	TOTOGACOTC ACACCTGCAG	CCGAATGGAA	CCCAAATTE	TACCTGAGGA	CTACCACCCC	CACGAGATTA	GGTTCTACGA CCAAGATGCT
25501	AGACCAATCC TCTOOTTAGG	COCCCOCCTA	ATGCCCTCGA TACCCCTCGA	TACCGCCTTC	GTYATTACCC CAGTAATGG	ACCCCCTTTA	TCTTGGCCAA	TTGCAAGCCA	TCAACAAAGC AGTTGTTTCG	CCCCCAAAA
25601	TTTCTGCTAC AAAGACGATG	GANAGOGACO	GGGCGTTTAC	TTGGACCCCC AACCTGGGGG	AGTCCGGCGA	CCTCGAGTFIG	CCAATCCCCC GOTTAGGGGG	COCCOCCOCO	GCCCTATCAG	CARCARCEO .

Figure 15P

pMRKAd5gag MBR682

ECOCOGNATA CHARGOCC CONTINUENCE AND ADDRESS AND ADDRES
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· Figure 1561

PMRKAd5gag MER682

GCAGAGCAAC CGTCTCTT*1 ATATCCAG*17 TATAGCTC*** GGACAGGG*1A CCTGTCCG***	AGNANTPA:A TETTTAAT" NTCTCTCC TAGAGGGGGG	TCCTTACC'''' AGGNATGGAC TGTTACCAG ACANATGGTC	Ctattectaat Gataagat''.	GCCTGCT() CGGACGACAC GTCAGCCCAC	ACCACAON TOOTOTOTY! TTACAGTTT'T AATOTCAAN	GTGGCCCCCA CACCGGGGGGG TACANARICA ATGTTTCC " AACAANTT" " TTGTTAAN "T ATTACCGAGGT
TACACCTCTC C CCCANGRATE N GGGCTCCTAG T TAGTTGAGC C ATCACTCG C	TAATAAATAC A ATTATTTATO T TACTTTTAAC A ATGAAAATTO T	AACACCACCC T TTOTOCTCCO A TCAATAACTC T AGTTATTGAG A	ACTOTACOOD C TGAGATOCCC O	AAGGCTCGCC G TTCCCGAGCGG C TCACCCTTGC G AGTGGGAACG C	TATAAAATGC ATATTTTACG GAGTATAATG CTCATATTAC	ACTATACTA TCATATTCAA CTATATTAAA GAYATAATTT CTGCTTCCAA GACGAACGTT TCTATGTCCC AGATACCCC AGATACCCC AGATACCCC AGATACCCC AGATACCCC AGATACCCC AGATACCCC AGATACCCC
ACTGGATTOTT TTACTTACAA CITTIGGATTOG GAAGCTTAAC CGCCCCTTGC GCGGGGACG	GTGCTGAGTA CACGACTCAT CCTTACCTGG GGAATGGACC	CATCAGAAAA GTAGTCTTTT COGACAGACC	AATTCAAGCA	TICTCTGCCT AAGAGACGGA CTAGGTTTAC GATCCAAATG	GCTANTRACT GCACCACTCT CGATTACTCA CGTGGTGAGA RTCAGCCAGG TGACACTACA CCGTCGGTCC ACTGTGATGT	TACTCOTTU
CHRICKTARIC ATTITIBETA TEANAGEAT GITTACECAG CAANTGGGTE	TCCCATCTCT ACGGTAGAGA CCAAGGCGAA GGTACCGCTT	TCAGCTACTC AGTCGATGAG AGACTTTTTC TCTGAAAAAG	GTTTATGAAC CAAATACTTG	ATACTAACGC TATGATTGCG GTACATAATC CATGTATTAG		TACCATGTAC ATGGTACATG GCFTTAGTCT CGAAACCAGA ACCACTAACT TGGTAATTGA CATTCCCCTG
GGACTCGGCG CCTGAGCCGC GACTCCGGTG CTGAGGCCAC TGATTCGGGA ACTAAGCCCT	AGATCTTTGT TCTAGAAACA CCCAAGCAAA GGGTTCGTTT	CTCTCCGAGC GAGAGGCTCG ACCGTAAACC TYGCATTTGG	CTACTGTGGG GATGACACCC	CTTTATTCTT GAAATAAGAA AGATGATTAG TCTACTAATC	CGCAGCTGAA GCGTCGACTT TATGCTATTT ATACGATAAA	TUTREGACNT ACAGGGTGTA TACAGTGGTG ATGTCAGTGTT TCGATTATCA TCGATTATGA
	GATTACATCA CTAATGTAGT TCTTCACCCG AGAAGTGGGC	ACGAGAGAAC TGCTCTCTTG CTACCGCCTG	ANAGGGGCAG TTTCCGCGTC	AACACTANGA TCGCCACCCA AGCGGTGGGT	ATGITACATT TACAATGIAA GIATACIGIT	ANANTACTITA CTATGCTAT GATACGATTA TAAGTTACAA ATTCAATGCT GGTCATTYCC CCAGTAAAGG
CCTAACTTAG GCCACAAGTG CGOTGTTCAC GGGAGAGCTT CCCTCTCGAA	CCTAACCCTG GGATTGGGAC AACGCCACCG TTGCGGTGGC	GAGTGAGTCT CTCACTCAGA TGCACCACAC	GTATTAGGCC CATAATCCGG	ATTCTCTGTC TAAGAGACAG AACGCTGGGG	CCAGCCTGTA GGTCGGACAT AAATTGGCAA TTTAACCGTT	TACTTTTCCA ATGANAGGT TGCTGCACTG ACGACGTGAC CTTAATTTAC GAATTAAATG
TCAATTTATT CCTAACTTTG ACGCGCTAAAA BGTTAAAATA GGATTGAAAG TGCGCTATTT CACTGTCGC GCCAAATA CTTTTCCCACGGGGACAGGG GGAAGTTCAC GAAAAGGGG GGAGATTCAC GAAAAAGGCAAAGGGGAAAGGT GCCCCTTAGCCAAATGGGGGAAAGGGAAAGGGAAAGGGAAAGGGAAAGGGAAAGGGAAAGGGAAAGGGAAAGGGAAAGGGAAAGGGAAAGGGAAAGGGAAAGGGAAAGGGAAAGGGAAAGGGAAAA	TTGCAACTGT AACGTTGACA CCATCCTGTA GGTAAGACAT			CCAACCCAA CACCTITITA GTCGAAAAAT	TTTTANGGNG CCAGCCTGTA AAAATTCCTC GGTCGGACAT CACAAAAAAA AAATTGGCAA GTGTTTTTGT TTTAACCGTT RAHION	CTTTTATCTA TGGCACTTTC ACCOTGNAG AGGAANAG TTCTTTTACG GAATAGGATT CTTATCCTAA
NCTATCCOSA TOTAL CONTROL CONTR	TCACTOTGAT A AGTGACACTA A GCTCCTATCG C			CTAGAATCGG GATCTTAGCC CATTTATTGT GTAAATAACA	AAAAGGTGGA TTTTCCACCT GCTTATTCGC CGAATAAGCG	TCAGTATTTT TGGAAMCAC ACCTTTTCTO TATTGAGGAA ATAACTCCTT ATTATAATTA TAATAATTAAT
CCTCCGGCC A GGAGGCCGG T TGCGCTGAA A ACGCGGACTT T CCCGGGCGC G GGGCCGCGCG C	CCCTGTGTTC 1 GGGACACAAG A ATATACTCOG G			AGA DET	Kprl GOTACCACCC CCATGGTGGG ATGAAAAGCT TACTTTTCGA	CCAGOSTIAA OOTCCCATT CAAATTGTG GTTTTAACAC GACUCGTCGAA AAAAGTTACATCG
27301 27401 27501	27601	27801	28001	28101	28301	28601 28601 28701 28801

Figure 1SR

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70697		MELECTRICARE CITIZENES CONTROLLOS TRESPESAS CONTROLARES CLCAAGGACC	TACAGTEGTA				AACAAGGTCA (GGTTGATGTC (SCHLINGOTH IN	
29001	TAACAGAGAT	TAACAGAGAT GACCAACACA ACCAACGCGG	ACCAACGGG	ACCAACACACA CCGCGGTAC CAGACTTACA		TUTACCACAA A	ATACACCCCA I	AGTITICINGCC 1	AAACAGTTAT	ACTSGGAT'AA TGACCCTA'IT
	ATTOICTA	ATTOTOTOTA CICAGIIGIGI	ואטרווירויי	ואירואסרויאווי		-		_	AACGCGCCCG /	ACCACCCA:TC
29101	CHICAGOCATO	CHICAGOCATO TOSTOCITOT	CCATAGGGGT	ATACAAACAT		•		_		TCGTV:CZ:TAG
29201	TATAGECCA	TATAGREECA TEATTORECT	ACACCCAAAC	ACACCCAAAC AATSTATSTAA TEFATAGATT GACCCAAFTG	TECATAGATT	-				TTANATCIACIA
, ,	ATATCAGGGT	ATATCAGGGT AGTNACACGA	TOTOGGETTE	TTACTACTIT	THACTACETT AGGTATETAN CETGCETCAC	-	TTTGTGTACA	ACAAAAGAGA	ATGTCATACT	AATTTACTO
		Whole was an annual contraction of the contraction			: Mi : Maladala la		ATTOGCTOCG	GTTCTCACA	TCGAAGTAGA	CTCCATTC A
10562	GTACTAAGGA	CTACTAAGGA GCTCAAAAT	ATAATGACTG	ATANTANCTE CONCONCE CANADANCAL CENCONTITO	CAANANACAC			CAAAGAGTGT	AGCTICATOR	GACGTAAG .T.
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29401	OCCTTCACAG	TCTATTIGGT	TTACGGATTT	GICACCCTCA	CCCTCATCTG		ACTUTOGREA TOCCONTAT		CCAGTICCATT	GACTOOCIN:T
	COGAAOTOTC		AGATAAACGA AATGCCTAAA	CNGTGGGNGT	GCGAGTAGAC GICLAMOTAG		EcoF			
			THE STATE OF THE S	CCCACTACAC	CCCAGTACAG GGACAGGACT	ATAGCTGAGC	TTCTTTAGAAT TCTTTAATTA		TOMATTITAC	TOTOACTTY
29501	CACACGCGAA	ACCEPTAGE OF		rengingaring aggregated centrecated integrated	CCTGTCCTGA		ANGNATCTTA AGAMATTAAT		ACTITEAAATO	ACACTONANA
10,00	CACACACAM			ATCITICATE DETECTORS COTTON AGES	CCTCCAAGCC		ATATICATICA GATTICACTCO	GATTCACTCG	TATATGGAAT	ATTCCAAGIT
10967	CHOCHOALTA			ACANGOGGET			TATAGTACGT	CTAAGTGAGC	ATATACCITIA	TAAGGTTCAA
							Pstl			
29701	OCTACAATGA		CTITICCGAAG	AAAAAGCGAT CTITCCGAAG CCTGGTTATA TCCAATCATC	TCCAATCATC	1CTGTTATEG	TOTTICTICE AND TACCATCITA	TACCATCITA		TATATCCC
	CGATGITACT		GAAAGGCTTC	GCACCANTAT	ACCTTAGTAG	ACCITAGTAG AGACAATACC ACAAGACGTC	ACAAGACGTC	ATCCTAGAAT		ATATMAGINI
20801	CONTRACATOR	_	CAATAGATGC	CATGAACCAC	CCAACTTTCC	CCANCITTICC CCGCGCCCGC TATGCTTCCA		CTCCNACANG	THOTHOCCGO	COCCULACIO
•	GGAACTGTAA					GENTANAGO GOCGCGGCG ATACGAAGGT		GACGITOTIC	AACAACGGCC GCCGAAAra	GCCCAAAAA 1
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		- Britannia	* Control of the Cont		AAATCAGCTA	ACCICIONIN AANICACIN CITTAATICIA ACAGGAGGA ATGACTGACA	ACAGGAGGAG.	ATGACTGACA	CCCTAGATOT	AGAAATTSGAC
10667	CUMBELLANIC	-			TITAGICGAT	THIAGHERAL GAANTAGAT TOTCCTCCTC TACTGACTEST	TOTOCTOCTO	TACTGACTGT		TCTTTACCTG
10001	GENATTATTA				CAGCGGCCGA	CAGCGGCCGA GCAACAGCGC	ATGAATCAAG	AGCTCCANGA	CATOOTTANC	TIGCACCAGE
	CCTTAATAAT	_		TCTGCGTCCC		Greggesor correspond	TACTTAGITIC	rcgaggrict	GTACCMATTIG	AACTITICGITCA
10101	CITABABATATA		CTCGTAMAGC	ACCCANGE	CACCTACGAC	AGTAATACCA	CCGGACACCG		AAGITICCCAA	CCANGCOTY
10100	COTTITICOCC		_	-	TECCETITICA GIEGATIGETE TEATTATEST		GCCTGTGGC	GUANTOCATO	TTCAACGGTT	GCT-N:CD-M:T
10201	CANATTOORS	_	GAGAMAAGCC		ACTCAGCACT	CATTACCATA ACTCAGCACT FRATAGAMAC	CCAAGGCTGC	ATTICACTICAC	CTTOTCAAGG	ACCTGARGAT
	CTTTANCCAC				TCACTCCTCA		GCTTCCGACG	TAAGTGAGTG	GAACAGTTCC	TOGACTICCTA
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30301	CTCTGCACCC		CCTOTOCOOT	CTCAAAGATC	TIATTCCCTT	CHENNAGATE TTATTECETT TARCTANTAN ANAMANTAN TRANSCATEN CITHETTANA	MANAMATAA	TAAAGCATCA	CTTACTTACA	RICAGITIANS
; ; ;	GACACCTOGG		GGACACGCCA	GAGTITICTAG	MTMGGGM	ANTANTICIE GEACACCECCA GAGTITICTAS AATAAGGIAA ATTGATTAIT TITITITATT ATTICGTAGT GAATGAATTT	TTTTTTTT	ATTTCCTAGT	CANTGAATTT	TAGTCARICG

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30401	ANATHICTOT	GCTCAAATAA	CAGCAGCACC	Technologia	CCTFCCAGCT	CHACTATAGG	AGCTTCCTCC	TOOCTOCAAA	CHITCICCAC	ANTCTAAATG
30501	GAATGICAGT				TATCTTCATG	THEFTICACAGA	TGAAGCGCGC	AACIACCGTCT		
	CTTACACTCA	AAGGAGGACA	AGGACAGGTA		ATAGAAGTAC	AACAACGTCT	ACTIVAGEG	TTCTGGCAGA	-	-
30601	GTATCCATAT	-	-	-	TITCITACIC	crecentier	ATCCCCAAT	OGGITTICAAG	-	TOGGGTACY :
	CATAGOTATA	CIGIGCCFFF	GCCAGGAGG	TTGACACGGA	AAAGAATGAG	GAGGGAAACA	TAGGGGGTTA	CCCAAAGTTC	TCTCAGGGGG	ACCCCATGAG
30701	Termococc	TATCCGACC	TETABITACE	TCCAATGGCA	TCCAATGCCA TGCTTGCCT	CAMANTROGC	AACTOCOLOTE	CTCTGGACGA	GCCCGCCAAC	CTTACCTCCY:
	AGAAACGCGG	ATAOCCTTOO	AGATCAATGG	AGGTTACCGT	ACCONCOCCA	GTTTTACCCG	TTGCCGGAGA	GAGACCTUCT	cceoccorra	GANTGGAG! 1
30801	MANATOTING	CACTOTORGE	CCACCTCTCA		GTCAAACATA	AACCTCGAAA	TATCTGCACC	CCTCACAGIT		CCCTAACTOT
30901	COCTOCCOCC	-		CAACACACTC	ACCARGOANT	רולאטעכניני	GCTAACCGTG	CACGACTECA	AACTTAGEAT	TOCCACCCAA
	CCGACGGCGG			GTTCTCTCAG	TOGTACGITTA	GIGICCGGG	CCATTCCCAC	CTCCTCACCT		ACCOTOCO I
31001	OGACCCCTCA	_	-	GCCCTGCAAA	CATCAGGCCC	CCTCACCACC	ACCGATAGCA	GTACCCTTAC	TATCACTOCC	TCACCCCCTT
	CCTOGGGAGT	GICACAGICT	TCCTTTCGAT	COCCACCTIT	GTACTCCOGG	CCACTCCTCC	TOGCTATOGT	CATOGGAATO	ATACTOACOG	AGTGGGGGAA
31101	TAACTACTOC	•	-	ACTTGAAAGA	CCCATTTAT	ACACAAAATG	GAMACTAGO	ACTABAGTAC	OCCOUNT	TOCATGTAL .
	ATTIGATORCO	•	-	TOANCIFICT	COCGINALIA	TGTGTTTTAG	CITITIGATICS	TOATTICATO	CCCCGAGGAA	ACCTACATENT
31201	AGACGACCTA			TOGITCCAGGI	GICACTATTA	ATAATACTTC	CTTGCAAACT	AAAGTTACTO	GAGCCTTOGG	TITIOATICA
	TCTGCTGGAT	•	-	ACCAGGTCCA	CACTCATAAT	TATTATCAAG	GAACCITTICA	TTTCAATOAC	CTCGGAACCC	MANCETANIC .
31301	CAAGGCAATA	•		GCACTAAGGA	THGATTCTCA	MACAGACCC	CTTATACTTO	ATOTTAGITA	TCCONTROAT	GCTCNAAAC
	Griccorrat	ACGITICAATT	ACATCGTCCT	CCTGATTCCT	AACTAAGAGT	THOTOTOGG	GAATATGAAC	TACAATCAAT	AGGCAAACTA	CONOTITION
31401	AACTAAATCT	-	CAGOGCCCTC	TITITATAAA	CTCAGCCCAC	AACTTCCATA	TTAACTACAA	CANAGOCCITY		CAGCTTCAA
	TTCATTTAGA	F.	GTCCCGGGAG	AAAAATATTT	GAGTCGGGTG	TYGAACCTAT	AATTGATGTT	GITTCCCGGAA	ATGAACAAT	GTCCAAGTT F
		Hardia								
31501	CAATTCCAAA	_			GOGITGAIGT	TTGACGCTAC	AGCCATAGCC	ATTAATGCAG	GAGATGGGCT	TOMATTHER
	CITAAGGITT	TTCOAACTCC	AATTCCATTC	GTGACGGTTC	CCCANCTACA	AACTGCGATG	TCGCTATCGG	TAATTACGTC	CTCTACCCGA	ACTTANACCA
31601	TCACCTAATO		CACCAMCAC - AAATCCCCTC		TRACCATOO	CCTAGAATTT	GATTCANACA	AGCTATOGE	TCCTAAACTA	CONCINCI
	ACTOGRAPTAC	CROOTTTOTO	TTTAGGGGAG	TITIGITIE	AACCGGTACC	CGATCTTAAA	CTAACTTIGE	TCCGATACCA	ACCATTTCAT	CCTTGACCCK
31701	TYACTITICA	CACCACAGGT		TAGGNAACAA	ANATANTGAT		TUTTENCONC	ACCAGCTCCA	TCTCCTAACT	GTACACTAAA
+	AATCAAAACT	GICOTOTICCA	CGGTAATGTC	Arccringth	TTTATTACTA	TTCGATTGAA	ACACCTRGGTG	TOCTCGAGGT	AGAGGATTGA	CATCTGATT
31801	TOCAGAGAAA	GATGCTAAAC	TCACTITICGE	CTTAACAAA	TOTEXCAGTE		TACACTTTCA	OTTITION	TTAAAGGCAG	TPIGGCTCCA
	Acoteterr	CTACGATITIG	AGTGAAACCA	DAATTGTTTT	ACACCGTCAG	TTTATGAACO	ATCTCANGE	CAAAACCGAC	AATTTCCGTC	AAACCCAGGT
31901	ATATCTOGAA	ATATCITICAA CAGITICAAAG	-	ATTATAAGAT	TTCACGAAAA		CTANACAATT	CCTTCCTGGA	CCCAGAATAT	TI. JAACTITA
	TATAGACCTT	PATAGACCTT GTCAAGTTTC	ACGAGTAGAA	TAATATTCTA	AACTGCTTTT	ACCTCACGAT	CATTICITA	GGAAGGACCT	CCCTCTTATA	ACCTICANAT
	and B	- A								
32001	GARATGGAGA	TCTTACTGAA		ATACANACGC	TOTTOGATIT	TOTTOGRAFIT ARGCTARC TATCAGCTTA TCCARATCT CACOUTARA CTGCCARARD	TATCAGCTTA	TCCAAAATCT	CACCOTANAA	CTCCCAAAAG
:	CITITACCICI	CITTACCTCT AGAATGACTT	CCCTCTCCGA	TATOTTTGCG	ACAACCTAAA	TACGGATTOG	ATAGTCGAAT AGGTTTTAGA	ACCTITITIOGA	GECCATETE	GACCOUTTING

Figure 15T

ринкадзана мекен2

CACACTOCA GTGTTRACCCAN** TAACGCGAY** CCACCACATA** GGTGGTGTA**	ANGRAGO " AGTONTATI TCACTATA GGSTANGATA CCGCTTCCT"	GCCACTCCGT CGCCGAGGCA 1 CACCCTGAY '	OCCICATORIC CCCICATORIC TOTICATATI TOTICATATA ACACATTAA PS!! TATACACTY ATATAGAG:	CACAGGCACA GTGTT:CGTGT WINDOWN CACTTCAGG GTGACGTCT: TGTCTCAAAA ACAGAGTTT F GTCATATTAAAA
ANACAGGAGA TITICATAC ANANGTATO TATAGCCCCA ATATCGGGGT TATACGGGGT TACACAGTCC	ATCHCTCAGG AACCCTCATC TTCCCAGTAG CTTAACCGGC GAATTGCCCG	TCCTGCCGCC ACGACGCGGG GCCAGCAGCG GTGTCGTCGC	GCTCATGGCG CGNGTACCGC TCTTTTGGCA AGAAAACCGT GCCCGCCGGC CGGCCGGCCG	GTTGGCACA CANCCGTGTT GTAAATCCCA CATTTAGGGT CGCCCCAAAG GCCCCCAAAG GCCCCCAAAG CCCCCCAAAG
GETACACAGG CCATGTGTCC CCTCTTACAC GCAGAATGTC CATTCAGTAG GTAAGTCATC ACACACAGAGA		GCGANTANAC CCCTTATTTG GTCCTCCGGG CAGGAGGCCC	TGCAAAGCGC TGTATCCAAA ACGTTCCCACG ACATAGGTTT CGCTGGACAT AAACATTACC GCGACCTGTA TTTGTAATGG AAACCAGCTG GCCAAAACCT TTTCCTCCAC CGCTTTTGGA	TCATATCAAT ACTATAGTTA CTGAATCAGC GACTTAGTCG AGTATAGCTC CANATGGAC GTTTACCTTG
TAGACTANAC TTTGCCACT NANGSOTETT ARTECATTTT TCAGTANAAA	11.5	COGREGORICE GCAGCAGCGC GCCACCACGA COTCOTCGCG CCCGCAACAT AAGGCGCCTT GGGCGTCGTA TTCCGCGGAA	·	ATCCTAGTOR TACGAGCAST TACCTAGTAG TACGAGCAST TCCCAGGGAA CAACCATTC AGGCTCCTT GTTGGTAAG GCACCAGGGS ATGATCTCC CCTCCTCCGC TACTAGGAGG TGTTCGTCGC TACTAGGAGG TGTTCGTCGCA TCACGAGTAC ACAACCAGCA TCACAGTACG
CACTANGCAT TANTGANTA ATTACTTANT GANANTTICA CITTINAAGT			AATCCCACAG TTAGGGTGTC CTCATAAACA GAGTATTTGT CCACCATGCT GGTGGTAGGA	
AAACCTETATA ACAN TACAT TOTTCATTATA TOTTCATTATA AAGTFAACCT	GGATCATANI GGATCATANI TTCTTANGETG AMGANTCCAC CCAMGTRICTG GGTCCACGAC	ANTEGREAT CAGATAGAS THAGCACGTA GECTATCCC CTCAGCGATG ATTCGCACCG GAGTCGCTAC TAAGCGTTATC	ACTOCAGEA ACACEACA TATTITAEAA TGACGTCGTG TEOTGGTOTT ATAACAAGTT CACAAGEGEA GCTAGATTAA GTTACGACCE GTGTTCGCGT CCATCTAATT CACCGCTGGG TAAACCTCTG ATTAAACATG GCGCCATCCA ATTTCGAAAC TAATTTGTAC CGCGGTAGGT	ACTCTTAACC TGACCATTGG TACATTGGTAT TTACATTGGG AATGTAAGCG ACCAGATCG TGGCTCTAGG
TCRETTITION TREPERSITE ACCARACTES GIRETTINITY CACANATAN	CTCACACACAAC GAGTGTCTTG AACAGACATA TTGTCTGTAT ATGTCCCTGT TACAGCGACA	ANTEGREGAT TYNGCACGTA CTCAGCGATG GAGTCGCTAC	ACCACCACAA TCCTGGTGTT CCTAGATTAA CCATCTAATT ATTAAACATG	Addeccadd TCTCGGGTC CCTCCGGCA TGANTGGCGCA TGANTTCAC CGCGGTCTGT
ACTTANACCE TEANTHREC TTCATGGGAC ANGITTCATC TACANAGTTC	CHTANTCANA GAATTAGTITT TATCATGGGT ATAGTACCCA ACTTAAGTIC TGAATTCAAG	GTAGAGTCAT. INTUCTICENT CNGGATAGGG CATCTCAGTA TTAGCACGTA GTCCTATCCC CAGTGGTCTC CTCAGCGATG ATTCGCACCG GTCACCAGAG GAGTCGCTAC TAAGCGTGTC Part		ATGACAGTTG TACTGTCACC ATTACAAGCT TAATGTTCGA CGTTGTGCAC GCACAGGTG GTACGACAGGTG GTACGACAGGTG GTACGACAGGTG
TCAGTTCAAA CTATGTCAAA GATACAGTAA CGTTTGTGTT GCAAACACAA	ANAGCATCA ANAGCATCA TTTTCGTAGT COGGCAGCTC GCCGTCGAG	CTACATOGGG GATGTACCCC TACAACATGG ATGTTGTACC	CNGCNCATA CITCATOTCAT GCCATCATAC CGGTNGTATG Kith COCTACCATA GCCATGOTAT	GACTGGACA CTGACCTTGT CTTCCTCAGG GAAGGACTCC ACGTACTCA TGCATTGAGT GATCCTACT CTAGGGATGA
TAACATTGTC ATTGTAACAG AGTGCATAGT TCACGTATGA AATAAAGAAT TTATTTCTTA	GCTTATACAG CCAATATGTC GCTGGCCTTA CGACCGGAAT ATAAACTCCC TATTTGAGGG	AAGTECAGGE TTCAGGTGCG Pall CCTGCAGGAA GGACGTCCTT	TCACTFAAAT AGTGAATTAA AACCCACGTG TTGGGTGCAC CACCACCTCC GTGGTGGGG	TCCCTTGGCC COTCCTACA GCACGTATGT AAGACCTCGC TTCTGGAGCG GGAGGTAGACC CCTCCATCTCG
32101 32201 32301	32401 32501 32601	32701	33001	33201 33301 33501

					Pigill						
	33601	CTCAACCAAA	ACCARGINGE	GGCGTGACAA	ACAGATOTGC (מוניוגרכממוכ י	ACTOCOCITY O	CTAPCCIAGAC A	TCTAGTAGIT	CATCATATAG	CACTUALICATOR COTON COTO
	11701	AAGCATYCAG	GUECCCCTO	GCTTEGGGTT				TCATAACATC (CACCACCGCA	GANTAAGCCA	CACCCARCC
		TTCOTAGGTC		CGAAGCCCAA				ACTATTOTAG (GTCGTCGCGT	CTTATTCGGT	GTGGGTCGLI
_	33801	ACCTACACAT	restretace	AGTCACACAC	GGGAGGAGCG	ממאמאמבדע				AGATTATCCA	AVACCTI TANA
		TCGATCTOTA	AGCAAGACGC	reagrerere	CCCTCCTCCC	CCTTCTCGAC	CTTCTTGGTA	CUMANAAAA	ANTAAGGTTT	TCTAATAGGT	TTTGGAGITT
		Bott	•							Contract of the Contract of th	
	33901	ATGAAGATCT		CGCGCINCCCC	TCCGGTGGCG		CTACAGCCAA			STEELS IN	CHCANGOCCE
		TACTTCTAGA	TAATTCACTT	CCCCGACGCG	ACCCCACCCC	ACCAGETEGA	GATGTCGGTT			ייייייייייייייייייייייייייייייייייייייי	מומו וווררניו
	34001	TECAMAGGE	AAACGGCCCT	CACGTCCAAG	TCCACCTAAA		TTCAGGGTGA			ACCACCTTCA	ACCATOCCCA
		AGOTTITICO	THICCGGGA	OTCCACGTTC	ACCTGCATTT	CCGATTTCGG	AAGTCCCACT	TAGACGAGAT		TCGTCGARGE	TOSTACOGET
	34101	ANTANTICIC	ATCTCOCCAC	CTTCTCAATA	TATCTCTAAG	CANATCCCGA	ATATTAAGTC			TCCAGAGCGC	CCTCCACCTT
		TTATTAAGAG	TAGAGCCGTG	GAAGAGTTAT	ATAGAGATTC	GTTTACCCCT	TATAATTCAG			AGGTCTCGCG	GCAGCTGGAA
	34201	CAGCCTCAAG	CAGCGAATCA	TGATTCCAAA	AATTCAGGTT	CCTCACAGAC	CHOTATAAGA			AAAAATACCO	CGATCCCGTA
		GICGGAGITIC		ACTAACGITT	TTAAGTCCAA	GGAGTOTCTO	GACATATTCT	AAGTETTCGC	CTTGTAAITIG	THTTATOOC	GCTAGGGCAT
	34301	COTCCCTTCC	CAGGGCCAGC	TGAACATAAT	CGTGCAGGTC	TGCACGGACC	AGCGCGGCCA	כידיכככככככ	-	ACANAGGAC	CCACACTUAL
		CCAGGGAAGC		ACTIGIATE	GCACGTCCAG	ACGIGCCTGG TCGCGCCGGT	TCGCGCCGGT	GAAGGGGCGG	recrirectae	TCTTTTCTIO	GGTGTGACTA
						Hindill	1111				
	34401	TATGACACGC		ATACTEGGAG 'C'TATECTAAC	CAGCGTAGCC	CCGATGTANG CTTGTTGCAT		GGGCGCGAT		AGGTGCTGCT	CAAAAAATC
	1	ATACTOTOCO		GATACGATTG	GTCGCATCGG	DOCTACATTC	GAACAACGTA	CCCGCCGCTA	TATTITIACGE	TCCACGACGA	GITTLITAGE
	34501	COCANAGEET	COCCCANANA	AGAAAGCACA	TCOTAGTCAT	CKTRCATRICAG	ATARAGGCAG	GTAAGCTCCG		AGAMANGAC	ACCAPITITION
	; ; ; ;	CCGTTTCGGA		retricerer	ACCATCAGTA	CGAGTACGTC	TATTTCCGTC	CATTCGAGGC		TC FITTICES	TOOTANAAAA
	14601	TCTCAAACAT	OPCTGCGGGT	TTCTCCATAA	ACACAMATA	ANATAACANA	MAACATTTA	AACATTAGAA		CAACAGGNAA	AACAACCC7'1
		AGAGITIGIA		AAGACGTATT	TGTGTTTTAT	TEATIGETE	TTTTGTAAAT	TTGTAATCTT		Griorccata	TTGTTGGGAA
	34701	ATANGCATAA	GACGGACTAC	OCCCATGCCG	GCGTGACCGT	AAAAAACTG	GTCACCOTGA	TTANANAGCA		CICCICGGIC	ATCTCCGGAG
	•	TATTCGTATT	CTGCCTGATG	CCCCTACGC	CCCACTCCCA	Trittenc	CAGTGGCACT	AATTTTTCGT		GACGAGCCAG	TACAGGCCTC
	34801	TCATAATOTA	AGACTCCCOTA	AACACATCAG	GPTGATTCAC	ATCGGTCAGT	GCTAANN GC	GACCGANATA		ATACATACCC	はこれはいらずから
		AGTATTACAT	TCTGAGCCAT	TICTCTAGTC	CAACTAAGTG	TAGCCAGTCA	CGATTTTTCG	CTGGCTTTAT		TATOTATOGG	CGICCCCAIC
	10901	AGACAACATT	ACAGCCCCCA	TACCAGGTAT	AACAANATTA	NTAGGAGAGA	ANANCACATA	AACACCTRIAA		GCCTAGGCAA	AATAGCACCC
		TCTOTTOTA		ATCCTCCATA	THOTTITANT	TATCCTCTCT	TITTICHCIAL	THETOGACTT	TTTTCCGACGA	COCATCCCTT	Trancerede
	35001	Tecogeteca	GANCAACATA	CAGCGCTTCC	ACAGCGGCAG	CCATAACAGT	CAGCCTTACC	ACTANAMAG	AAAACCTATT	MAMANAACAC	CACTCGACA
		ACCOCCAGOT	-	GTCGCGAAGG	TGTCGCCGTC	GGTATTGTCA	CICCCAAICG	TCATTTTTC	TTTTCGATAA	TITITION	GIGAGCIUM
	15101	CONTRACTACE	. CAATCAGTCA	CAGTGTMM	AAGGGCCAAG	TGCAGAGCGA	CTATATATAG	GACTAAAAAA		GITTAAAGICC	ACAAAAAAA
		CCOTOGTCGA		GTCACATTTT	Trecedente	ACGICICACT	CATATATAT	CICATIFIE	ACTGCATTGC	CAATITICAGG	
	15201	CCCAGAAAC	COCACOCOAA	CCTACGCCCA	GANACCANAG	CCANNANCC	CACAACTTCC	TCAAATCGTC	ACTICCGITIT	TCCCACGITA	
		GGCTCTTTTG		CGATCCCCCT	CTTTGCTTTC	GGTTTTTGG	GTGTTGAAGG	AGTITAGCAG	TOARGCAAA AGGGTGCAAT	AGGGTGCAAT	GCAGTGAAGG

Figure 15V

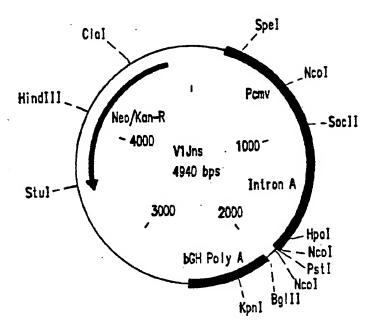
35301	CATTITIADA			TACAAGTTAC	Trenceta	AACCTACCTIC ACCCGCCCCG	ACCCGCCCCG	TTCCCACGCC		TCACAAACTC
	GTANAATTCT	TTTGATGTTA	ACCCTICACT	ATGITICAATG	ATGITTCAATG AGGGGGGATT	TTGGATGCAG	אאפרטאפר	MARSINCE	GER COLLEC	ACTOR
						Pac				•
•							Fenfil			
35401	CACCCCTCA	TTATCATATT	GGCTTCAATC	CAMMTANGG	TATATTATTG	ATKIATCITAA		GGATCTGCGA	CGCCAAGGCTG	GATCGCCTT.
	GTOGGGGAGT	AATAGTATAA		GTTTTATTCC	ATATAATAAC	TACTACANTT	AATTCTTAAG	CCTAGACGCT	OCCICTOCOAC	CTACCOOMO
35501	CCCATTATOA	Trefrences	Treesesses	ATCGGGATGC	CCGCGTTGCA	COCCATGCTO	TCCAGGCAGG	TAGATGACGA	CCATCAGGGA	CAGCTTCAAG
i i	GCOTAATACT	-		TAGCCCTACG	GGCGCAACGT	CCCCTACGAC	AGGICCGICC	ATCTACTOCT	GGTAGTCCCT	GICONAGITIC
35601	GCCAGCAAAA	GGCCAGGAAC	CUTAAAAAGG	CCCCOTTCCT	GGCGTTTTTC	CATAGGCTCC	GCCCCCCTGA	CONGCATCAC	ANAMATECIAC	GCTCAAGTCA.
	COOLCOPPIN	CCGCICCTIG	_		CCCCANNAG	CITATCCCAGG	CCCCCCCACT	OCTCGTAGTO	TTTTAGCTO	CCACTTCAGT
35701	CAGOTOCCOA	AACCCCACAG	GACTATAAAG	ATACCAGGCG	TTTCCCCCTC	GAAGCTCCCT	CGROCOCICT	CCTOTTCCGA	CCCTGCCGCT	TACCCCATAC
	CTCCACCOCT	TYGGGCTGTC	CHGATATTTC	TATOGICCGC	ANAGEGGGAC	CTTCGAGGGA	CCACCCCAGA	CCACAAGGCT	CULACGGCGA	ATOCCTATY:
35801	CTOTCCOCCT	TICTCCCTTC	OGGNAGCGTG	GCGCTTTCTC	ATAGCTCACG		CTCANTTACGG	TOTAGGTCGF	Tegetecano	CTREGGETTED.
	GACAGGCGGA	AAGAGGGAAG	CCCTTCGCAC	CCCCANAGAG	TATCGAGTGC	GACATCCATA	CARTICAACIC	ACATCCAGCA	ACCCAGGITC	GACCCGACN.
35901	TOCACOAACC	CCCCGFTCAG	CCCGACCGCT	GCGCCTTATC	CGGTAACTAT	CGTCTTGAGT	CCAACCCGGF	AAGACACGAC	TTATCOCCAC	TOOCAGCAG
	ACCTOCITION	GOGGCAAGTC	OGGCTGGCGA	CGCGGAATAG	GCCATTGATA	GCAGAACTCA	GGTTGGGCCCA	Treferecte	AATAGCCCTC	ACCONCORC
36001	CACTOOTAAC	AGGATTAGCA	GAGCGAGGTA	TOTAGGEGGT	GCTACACAGT	TETTISANGTO	GTGGCCTAAC	TACCOCTACA	CTAGAAGGAC	AGTATTTGGF
	CTCACCATTC				CGATCTCTCA	AGAACTTCAC	CACCOGATIG	ATGCCGATGT	DATETTECTO	TCATANACCA
36101	ATCTOCGCTC	TOCTGNAGCC	AGITACCINC	CCAAAAAGAG	TRESTACCIE	THEATCCOCC	ANACANACCA	CCCCTOGTAG	COORGITTE	THY GITTINGC.
!	TAGACGCGAG		f CAATCGAAG	CCTTTTCTC	NACCATCGAG	AACTAGGCCG	Tricitroct	GGCGACCATC	GCCACCANA	AAACAAACTTI
36201	ACCAGEAGAT	TACGCGCAGA	AAAAAAGGAT	CTCAAGAAGA	TCCTTTGATC	TITICINCGG	GGTCTGACGC	TCAGTOGNAC	GAMACTCAC	CTTANGGGA'
	TCOTCOTCTA	ATGCGCGTCT	TTTTTTCCTA	GAGTTCTTCT	ACCANACTAG	MANGATOCC	CCAGACTYXCG	AGTCACCTTO	CTTTTOAGTO	CAATTCCCTIV
36301	THOOTCATO	AGATTATCAA	AAAGGATCTT	CACCTAGATC	CTTTTAAATC	ANTCTANAGE	ATATATGAGT	ANACTROOPE	TOACAGTTAC	CNATCCITA
	MACCAGTAC	TCTAATAGIT	TTTCCTAGAA	GTGGATCTAG	GAMMATTTAG	TTAGATTTCA	TATATACTCA	TITCAACCAO	ACTOTICAATO	GTTACGNATT
36401	TCAGTGAGGC	ACCTATCTCA	OCCUPATION OF	TATTTCGTTC	ATCCATAGTT	GCCTOACTCC	ccorcerctA	GATAACTACG	ATACGGGAGG	GCTTACCATY:
	AGICACTCCG	TOGATAGAGT	COCTAGACAG	ATNANGCANG	TACCTATCAA	CCAGACTGAGG	GGCAGCACAT	CTATTCATCC	TATOCCCTCC	CCNATCKITAG
36501	TOCCCCAOT	GCTGCAATGA	TACCCCGAGA	CCCACGCTCA	CCGGCTCCAG	ATTTATCAGE	AATAAACCAG	CCACCCCCAA	GCCCCAGCG	CACIANGTOGT
	ACCOGGGTCA	CGACGITIACT	ATGCCCCTCT.	GCGTGCGAGT	CCCCARGIC	TAMTAGICG	TTAITTIGGIC	GOTCOCCUT	CCCOCCICOC	CHCITICACCA
36601	CCTOCAACTT	* FATCCOCCTC	CATCCAGTCT	ATTAATTOTT	GCCGGGAAAC	TANASTAAGT	AGTTCCCCAG	TTAATAGTTT	GCGCAACGIT	GITTGCCATTG
	GCACCTTCAA	ATAGGCCGAG	GTAGGTCAGA	TAATTAACAA	CGCCCTTCG	ATCTCATTCA	TCANGCGGTC	AATTATCAAA	CCCOTTCCAA	CAACGGTAAC
36701	CTACAGGCAT	CGTOGTOTCA	COCTCGTCGT	TTOOTATGO	TTCATTCAGC	TOCOGETICOC	AACGATCAAG	GCCAGTTACA	TGATCCCCCA	TOTTOTOCAA
	GATOTCCOTA			AACCATACCG	AAGTAAGTCG	ACCCANGGG	TTGCTAGTTC	COCTCAATGT	ACTAGGGGGT	ACAACACGTT
				-						
36801	AAAAGCGGTT	MACTICETTES	GTCCTCCGAT	GTCCTCCGAT CCTTGTCAGA			ATCACTCATG	OFFRATOCCAG	CACTGCATAA	TICTCTTACT
	TTTTCCCCAA	TCGAGGAAGC	CAGGAGGCTA	GCAACACTCT	TCATTCAACC	COCCITCACAA	TACTICACTAC	CANTACCOTC	GTCACGTATT	ANGAGANTGA
36901	CACATGCCAT	CCGTAAGATG	CHTTTCTGTG	ACTGGTGAGT	ACTUANCOAN	CACTANGACT	CTTATCACAT	TOTOCCGACC	CTCAACGAGA	TCCCCGGCGT ACGCGCCGCA
:	10001000									

Figure 15W

PMRKAd5gag MER682

CARCACGOS FANTACCOCO CCACATARCA GAACTITAAA AGTECTEATE ATTRAAAAA GATCTITGOS OCGAAAACTE TCAAGGATET TACRETETT GITGTGCCCT ATTATGGCGC OCTOTATCOT CITEAAATTI TEACGACTAG TAACCTITIG CAACAAGCCC COCTITIGAG AGTECTAGA ATGGCGAAAA GAGATCCAGT TCGATGTAAC CCACTCGTGC ACTCAACTAA TCTITAAATTI CATTTACTIT CACCAACGT TCTICGAAG CAAAAAAAAA	CTCTAGGICA AGCTACATTG GOTGAGGACG TXGGTTGALT AGAACTGATA GAAAATGAAA GTXTGCGAA AGACCACTC GTTTTTGTCC TTGCGTTTTTA GCCCAAAAA AGGGAATAAG GGGAACACGG AAAAGTTGAACTTGAACTTGAT CTTCCTTTTT CAATATTATT GAAGCATTA TCAGGGTTAT TGTCTCATGA CGGGGATTTT TCCCTTAATT GCCCCAATA AGACCAATA AGACCAATA ACAGAGTTATT TCCCTTAATC CTGCCTATGC TTTACAACTT ATGAGTTATTA AAAGAGTAGTA	GCGGATACAT ATTIGAATOT ATTIAGAAA ATAAACAAAT AGGGGTTTCG GCGTGTAAA GGGGTTTTCA GCGACGACG GTGTAAGAAA CCATTATAAA GGCGTAATAAA GGCGTATTAA GGCGTATTACA CGGGGACTG CAGATTCTTT GGTAATAATA GGCCTATTAA CGGGGACTG CAGATTCTTT GGTAATAATA GGCCTATTACA CGGGGACTG CAGATTCTTT GGTAATAATA GGCCTATTACA CGGTGGACTG CAGATTCTTT GGTAATAATA	
TCAAGGATC AGTTCCTAG	GTTTTTGTC TCAGGGTTA AGTCCCAAT	GAGATICIT	ID NO: 27) ID NO: 28)
SAVAACTO STITTIGAG	ACCCACTC AGCATTTA ACCTAAAT	CACCTGAC	NT (SEQ
200	\$ 5 E	# 300 ₩ 000	T TA
GITCTTCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	CAATATTAT GTTATAATA	CCCGAAAAAG GGGCTTTTTC Feetti	TUCGAATTC
ATTCCANAAC TAACCTTTTG	GAAAATGAAA CTTCCTTTTT GAAGGAAAAAA	GCGTGTAAAG GCGTGTAAAG	Bamell AAGAATTEGA TUCI TTCTTAACCT AGGG
AGTECTEATE TEACGAGTAG TEPTEAGEAT	AGAACTUCTA TACTCATACT ATTACTATEA	AGGG:TTTCG TCCCCAAGGC	TITEGETETTE
GAACTTTAAA CTTGAAATTT ACCGAAGTGA	TCGGTTCACT AAATGTTGAA TTTACAACTT	ATAAACAAAT TATTTGTTTA	CACGAGGCCC
CCACATAGGA GGTGTATCGT CCACTCGTGG	GGTGAGGACG GGCGACACGG CCGCTGTGCC	ATTTAGAAAA TAAATCTTTT	ATACGCGTAT TATCCGCATA
TAATACCGCG ATTATGGCGC TCGATGTAAC	AGGGAATAAG TCCCTTATTC	ATTTGAATGT TAAACTTACA	CATGACATTA ACCTATAAAA ATAGGCGTAT CACGAGGCCC TITCGTCTTC AAGAATTGGA TYCGAATTCT TAAT (SEQ ID NO: 27) GTACTGTAAT TGGATATTTT TATCCGCATA GTGCTCCGGG AAAGGAGAAG TYCTTAACCT AGGCTTAAGA ATTA (SEQ ID NO: 28)
CAACACGGGA GTTGTGCCCT GAGATCCAGT	CTCTAGGTCA GCCGCAAAAA CGGCGTTTTT	GCCGATACAT CGCCTATGTA	CATGACATTA ACCTATAAAA ATAKGCGTAT CACGAGGCC TITICGTCTTC AAGAATTGGA TICGAATTCT TAAT (SEQ ID NO: 27) GTACTGTAAT TGGATATTTT TATCCCGCATA GTGCTCCAGG AAAGCAGAAA TICGAATTCT TAAT (SEQ ID NO: 27)
37001	37201	37301	37401

Figure 15X



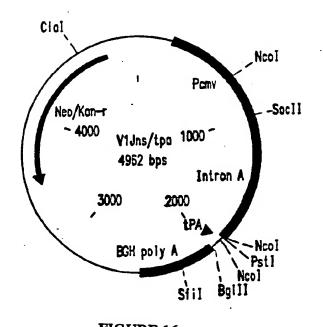


FIGURE 16

AGATOT	ACCATGGCCCC	CATCTCCCCATTGAGACTGTGCCTGTGAAGCTGAAGC	CTGGCATGGATGGCCCCAAGGTGAA
Balll		olleSerProlleGluThrVolProVolLysLeuLysP	
	1	10 `	20

GCAGTGGCCCCTGACTGAGGAGAAGATCAAGGCCCTGGTGGAAATCTGCACTGAGATGGAGAAGGAGGGCAAAATCTCCA sGinTrpProLeuThrGluCluLyslleLysAloLeuVolGluIleCysThrGluMelGluLysGluGlyLysIleSerL 30 40 50

AGATTGGCCCCGAGAACCCCTACAACACCCCTGTGTTTGCCATCAAGAAGAAGAAGACCTCCACCAAGTGGAGGAAGCTGGTG
yslieGiyProGiuAsnProTyrAsnThrProVoiPheAiolieLysLysLysAspSerThrLysTrpArgLysLeuVoi
60 70

GACTTCAGGGAGCTGAACAAGAGGACCCAGGACTTCTGGGAGGTGCAGCTGGGCATCCCCCACCCCGCTGGCCTGAAGAA AspPheArgGluLeuAsnLysArgThrGlnAspPheTrpGluVolGlnLeuGlyIleProHisProAloGlyLeuLysLy 80 90 100

GAAGAAGTCTGTGACTGTGCTGGCTGTGCGGGATGCCTACTTCTCTGTGCCCCTGGATGACGACTTCACGAAGTACACTG slyslysSerVoIThrVoILeuAloVoIGIyAspAloTyrPheSerVoIProLeuAspGluAspPheArgLysTyrThrA 110 120 130

CCTTCACCATCCCCTCCATCAACAATGAGACCCCTGGCATCAGGTACCAGTACAATGTGCTGCCCCAGGGCTGGAAGGGC
IoPheTnrlleProSerileAsnAsnGluThrProGlylleArgTyrGlnTyrAsnVolLeuProGlnGlyTrpLysGly
140 150

TCCCCTGCCATCTTCCAGTCCTCCATGACCAAGATCCTGGAGCCCTTCAGGAAGCAGAACCCTGACATTGTGATCTACCA SerProAlollePheGInSerSerMetThrLyslleLeuGiuProPheArgLysGInAsnProAsplleVollleTyrGI 160 170 180

GTACATGCCTGCCCTGTATGTGGGCTCTGACCTGGAGATTGGGCAGCACGACCAAGATTGAGGAGCTGAGGCAGCACC
nTyrMeiatololeuTyrVoiGiySerAspleuGiulleGlyGinHisArgThrLysIleGluGluLeuArgGinHisL
190 200 210

TGCTGAGGTGGGGCCTGACCACCCCTGACAAGAAGCACCAGAAGGAGCCCCCCTTCCTGTGGATGGGCTATGAGCTGCAC euleuArgTrpGlyLeuThrThrProAspLysLysHisGInLysGluProProPheLeuTrpMetGlyTyrGluLeuHis 220 230

CCCGACAGTGGACTGTGCACCCATTGTGCTGCCTGAGAAGGACTCCTGGACTGTGAATGACATCCAGAAGCTGGTGGG
ProAspLysTrpThrVoIGinProIIeVoILeuProGluLysAspSerTrpThrVoIAsnAspIIeGInLysLeuVoIGI
240 250 260

CAAGCTGAACTGCGCCTCCCAAATCTACCCTGGCATCAAGGTGAGGCAGCTGTGCAAGCTGCTGAGGGCACCAAGGCCCC
yLysLeuAsnTrpAloSerGinlieTyrProGlylieLysVolArgGinLeuCysLysLeuLeuArgGlyThrLysAloL
270 280 290

FIGURE 17A

TGACTGAGGTGATCCCCCTGACTGAGGAGGCTGAGCTGGAGCTGGAGCTGGAGAACAGGGAGATCCTGAAGGAGCCTGTGCAT EUThrGluVollleProLeuThrGluGluAlaGluLeuGluLeuAlaGluAsnArgGluIleLeuLysGluProVolHis 300 310

GCGGTGTACTATGACCCCTCCAAGGACCTGATTGCTGAGATCCAGAAGCAGGGCCAGGGCCAGTGGACCTACCAAATCTA GlyVolTyrTyrAspProSerLysAspLeulleAloGlulleGlnLysGlnGlyGlnGlyGlnTrpThrTyrGlnlleTy 320 330 340

CCAGGAGCCCTTCAAGAACCTGAAGACTGGCAAGTATGCCAGGATGAGGGGGGCCCCACACCAATGATGTGAAGCAGCTGA rGInGIuProPheLysAsnLeuLysThrGiyLysTyrAloArgMeLArgGlyAloHisThrAsnAspVolLysGInLeuT 350 360 370

CTGAGGCTGTGCAGAAGATCACCACTGAGTCCATTGTGATCTGGGGCAAGACCCCCAAGTTCAAGCTGCCCATCCAGAAG hrGluAloVolGlnLyslleThrThrGluSerlleVollleTrpGlyLysThrProLysPheLysLeuProlleGlnLys 380 390

GGTGAAGCTGTGGTACCAGCTGGAGAAGCAGCCCATTGTGGGGGCTGAGACCTTCTATGTGGCTGGGGCTGCCAACAGGG uVolLysleuTrpTyrGInLeuG1uLysG1uProlleVolG1yA1oG1uThrPheTyrVolA1oG1yA1oA1oAsnArgG 430 440 450

AAGACTGCCCTCCAGGCCATCTACCTGGCCCTCCAGGACTCTGGCCTGGAGGTGAACATTGTGACTGCCTCCCAGTATGC LysThr AloLeuGinAloileTyrLeuAloLeuGinAspSerGiyLeuGiuVolAsnIieVolThr AloSerGInTyrAi 480 490 500

CCTGGGCATCATCCAGGCCCAGCCTGATCAGTCTGAGCTCTGAGCTGGTGAACCAGATCATTGAGCAGCTGATCAAGAAGG ©LeuGiylielieGinAloGinProAspGinSerGiuSerGiuLeuVolAsnGinIlelieGiuGinLeulieLysLysG 510 520 530

AGAAGGTGTACCTGGCCTGGCCTGCCCACAAGGCCATTGGGGGCAATGAGCAGGTGGACAAGCTGGTGTCTGCTGGC
IULysVolTyrLeuAloTrpVolProAloHisLysGlylleGlyGlyAsnGluGlnVolAspLysLeuVolSerAloGly
550

ATCAGGAAGGTGCTGTTCCTGGATGGCATTGACAAGGCCCAGGATGAGCATGAGAAGTACCACTCCAACTGGAGGGCTAT

1 leAr gl ysVoil euPheleuAspGiylleAspLysAioGInAspGluHisGluLysTyrHisSerAsnTrpAr gAloMe
560 570 580

FIGURE 17B

CGCCTCTGACTTCAACCTGCCCCCTGTGGTGGCTAAGGAGATTGTGGCCTCCTGTGACAAGTGCCAGCTGAAGGGGGAGG tAloSerAspPheAsnLeuProProVolVolAloLysGIuIleVolAloSerCysAspLysCysGInLeuLysGIyGluA 590 600 610

GCTGTGCATGTGGCCTCCGGCTACATTGAGGCTGAGCTGATCCCTGCTGAGACAGGCCAGGAGACTGCCTACTTCCTGCT AlovolHisVolAloSerGlyTyrIleGluAloGluVollleProAloGluThrGlyGlnGluThrAloTyrPheLeuLe 640 650 660

GAAGCTGGCTGGCAGGTGGCCTGTGAAGACCATCCACACTGCCAATGGCTCCAACTTCACTGGGGCCACAGTGAGGGCTG - uLysLeuAloGlyArgTrpProVolLysThrlleHisThrAloAsnGlySerAsnPheThrGlyAloThrVolArgAloA 670 680 690

CCTGCTGGTGGCCTGGCATCAAGCAGGAGTTTGGCATCCCCTACAACCCCCAGCGGGTGGTGGCCTCCATGAAC IoCysTrpTrpAloGlylleLysGlnGluPheGlylleProTyrAsnProGlnSerGinGlyVolVolAloSerMelAsn 700 710

AAGGAGCTGAAGAAGATCATTGGGCAGGTGAGGGACCAGCCTGAGCACCTGAAGACAGCTGTGCAGATGGCTGTGTCAT LysGluLeuLysLysItelleGlyGlnVolArgAspGlnAloGluHisLeuLysThrAloVolGlnMetAloVolPheli 720 730 740

CCACAACTICAAGAGGAAGGGGGCATCGGGGGCTACTCCGCTGGGGAGAGGATTGTGGACATCATTGCCACAGACATCC
eHisAsnPheLysArglysGlyGlylleGlyGlyTyrSerAloGlyGluArglleVolAsplleIleAloThrAsplleG
750 760 770

AGACCAAGGAGCTCCAGAAGCAGATCACCAAGATCCAGAACTTCAGGGTGTACTACAGGGACTCCAGGAACCCCCTGTGG
InThrLysGTuLeuGInLysGIniTeThrLysTieGInAsnPheArgVoITyrTyrArgAspSerArgAsnProLeuTrp
780 790

AAGGCCCCTGCCAAGCTGCTGTGGAAGGGGGAGGGGGTGTGGTGGTGATCCAGGACAACTCTGACATCAAGGTGGTGCCCAG LysGiyProAiolysLeuLeuTrpLysGiyGiuGiyAioVoiVoiIleGinAspAsnSerAspIleLysVoiVoiProAr 800 810 820

AAACCCCCCCCACATC; (SEQ ID NO: 3)

Xx Bg/11 (SEQ ID NO: 4)

FIGURE 17C

GATCACCATGGAATGAAGAGAGGCTCTGCTGTGTGCTGCTGTGTGGAGCAGTCTTGGTTTGCC
MetaspalometlysArgGlyteuCysCysVolLeuLeuLeuCysGlyaloVolPheVolSerP
-25
-26

FIGURE 18

WT	- ATG GGT GGC AAG TGG TCA AAA CGT AGT GTG CCT GGA TGG TCT -42
OPT	- ÁTG GÁC ÁSÁ TÁG TÁG TÉC ÁÁG AĞG TCC ÉTĞ ČĆC ĞĞC TĞĞ TĆC M G G K W S K R S V P G W S -14
WT	- ACT GTA AGG GAA AGA ATG AGA CGA GCT GAG CCA GCA GCA GAT -84
OPT	- ÁCC GTG ÁGG GÁG ÁGG ÁTG ÁGG AGG GCC GÁG CCC GCC GÁC T V R E R M R R A E P A A D -28
WT [*]	- AGG GTG AGA CGA ACT GAG CCA GCA GCA GTA GGG GTG GGA GCA -126
OPT	- ÁĞĞ ĞTĞ ÁĞG AĞG ÁČC ĞÂĞ ČČC ĞČC ĞČC ĞTĞ ĞĞC ĞTĞ ĞĞC ĞCC R V R R T E P A A V G V G A -42
WT	- GTA TCT CGA GAC CTG GAA AAA CAT GGA GCA ATC ACA AGT AGC -168
OPT	- GTG TCC AGG GÁC CTG GÁG ÁÁG CÁC GGC GCC ÁTC ÁCC TCC TCC V S R D L E K H G A I T S S -56
₩T	- AAT ACA GCA GCT ACC AAT GCT GAT TGT GCC TGG CTA GAA GCA -210
OPT	- ÁÁC ÁCC GCC ÁCC ÁÁC GÁC GÁC TGC GCC TGG CTG GÁG GCC N T A A T N A D C A W L E A -70.
WT .	- CAA GAG GAT GAG GAA GTG GGT TTT CCA GTC AGA CCT CAG GTA -252
OPT	- CÁG GÁG GÁC GÁG GÁG GTG GGC TTC CCC GTG ÁGG CCC CÁG GTG Q E D E E V G F P V R P Q V -84
WT	- CCT TTA AGA CCA ATG ACT TAC AAG GGA GCT GTA GAT CTT AGC -294
OPT	- CCC CTG ÁGG CCC ÁTG ÁCC TÁC ÁÁG GGC GCC GTG GÁC CTG TCC P L R P M T Y K G A V D L S -98
WT	- CAC TIT TTA AAA GAA AAG GGG GGA CTG GAA GGG CTA ATT CAC -336
OPT ·	- CAC TTC CTG AAG GAG AAG GGC GGC CTG GAG GGC CTG ATC CAC H F L K E K G G L E G L I H -112
WT	- TCA CAG AAA AGA CAA GAT ATC CTT GAT CTG TGG GTC TAC CAC -378
OPT	- TCC CAG AAG AGG CAG GAC ATC CTG GAC CTG TGG GTG TAC CAC S Q K R Q D I L D L W V Y H -126
WT	- ACA CAA GGC TAC TTC CCT GAT TGG CAG AAC TAC ACA CCA GGG -420
OPT	- ACC CAG GGC TAC TTC CCC GAC TGG CAG AAC TAC ACC CCC GGC T D G Y F P D W D N Y T P G -140

FIGURE 19A

WT		CCA	11	111	11	11	11	-11	111	11	11	111	111	111	111	•	462
OPT	•	ĊĊC P	39 9 6				CCC P	CTG L	ACC T	TTC F	39 9 9	TGG W	TGC C	TTC F	AAG K	,	154
₩Т		11	.11	11	11	111	11	II	111	11	11	411	Π	Ш	GAA 		504
OPT	-	ĊŤG L	ĠŤG V	ĊĊC P	ĠŤG V	GÁG E	CCC P	GAG E	aag K	GTG V	GAG E	GAG E	GCC A	aac N	GAG E		-168
WT		GGA 	111	111	111	111	- 11	1	111	- 11	111	- 1			11		- 546
OPT '	•	ogc G	ĠÀĠ	ÁÁĊ N	AAC N	TGC C	CTG	CTG L	CAC	CCC	ATG M	TCC S	CAG Q	CAC H	GGC G		-182
WT		ATA	111	111	11	111	$\Pi\Pi$	11	111		-111	111	-111	11	-11		-588
OPT	•	ATC	GAG E	GAC	CCC	GAG	AAG	GAS	GTG	CTG	GAG E	TGG	AGG R	TTC F	GAC D		-196
WT .		AGC	: AAG		GCA	П	CAT		GTG			GAG		CAT	CCG		-630
OPT		TCC	. AAG	CTG	GCC	TTC	CAC	CAC	GTO	GCC	AĞG A R	GAG	CTG	CAC	CCC		-210
WT		- GAG		TAC	AAG		TGC	TGA	(2	EQ I	D NC	:30)					-651
O PT		 GAG	TAC	TAC	AAG	GAC	TGC	TAA) (c	conta SEQ I	inec D NO	l wii : 10)	hin	SEC	D NO	:9)	-216

FIGURE 19B

VIJns/nef

CATGGGTCTTTTCTGGGTCACCGTCCTTGAGATCTGCCACC ATG GGC GGC AAG TGG TCC AAG AGG TCC GTG CCC .

SrfI B9111

CAC CCC GAG TAC TAC AAG GAC TGC TAA AGCCGGGGGAGAICIGCTGGCC77C7AG77GCCAGC (SEQ 1D NO: 38)

H P E Y Y K D C * (Contained within SEQ 1D NO: 10:

V1Jns/nef(G2A.LLAA)

Psti Catgastettttelgegteaccetecttgaga<u>tet</u>gecace atg gcc ggc ang tgg tcc aag agg tcc gtg ccc . M A G K W S K R S V P

Srff BgIII CAC CCC GAG TAC TAC AAG GAC TGC TAA AGCCCGGGAGAICIGCTGCCTTCTAGTTGCCAGC (SEQ ID NO: 39) H P E Y Y K D C * (contained wilhin SEQ ID NO:14)

/lJns/tpanef & VlJns/tpanef(LLAA)

Psti Catesgretiti<u>ciecag</u>teacestecttatatetagateace atg gat gea atg ang aga ggg ctc tgc tgt gtg M D A M K R G L C C V

SPFI BGILI

CAC CCC GAG TAC TAC AAG GAC TGC TAA AGCCCGGGCAGGICTGCCTGTGCCTAGTTGCCAGC (SEQ ID NO: 40)

H P E Y Y K D C * (contained withon seq id no: 16) CTG CTG CTG TGT GGA GCA GTC TTC GTT TCG CCC AGC GAG AIC TCC AAG AGG TCC GTG CCC ...

FIGURE 20

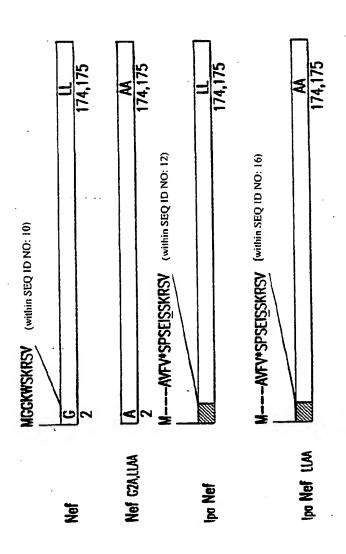


FIGURE 21

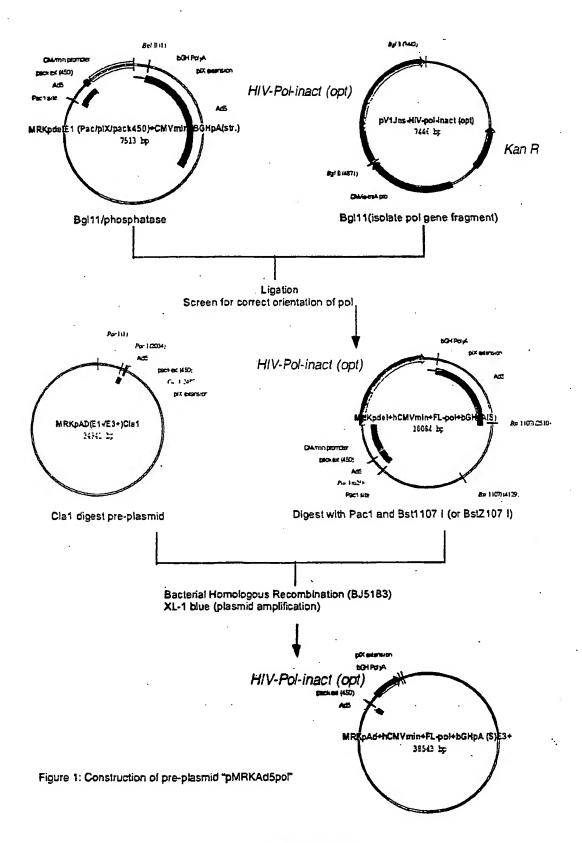


FIGURE 22

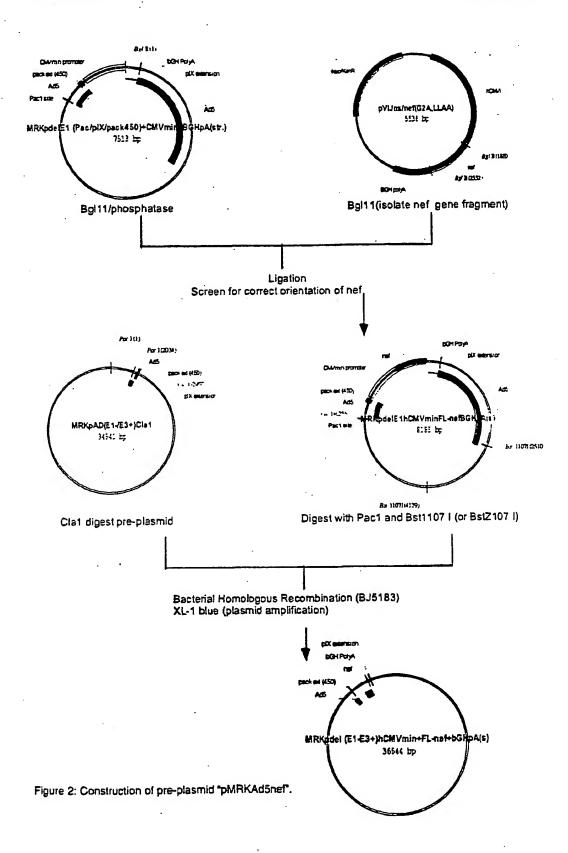


FIGURE 23

Comparison of Clade B vs. Clade C Anti-gag T Cell Responses in Clade B HIV-Infected Subjects

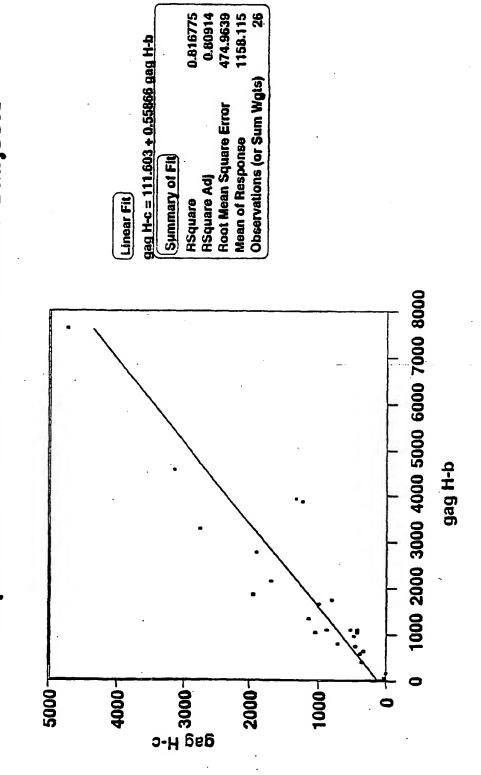
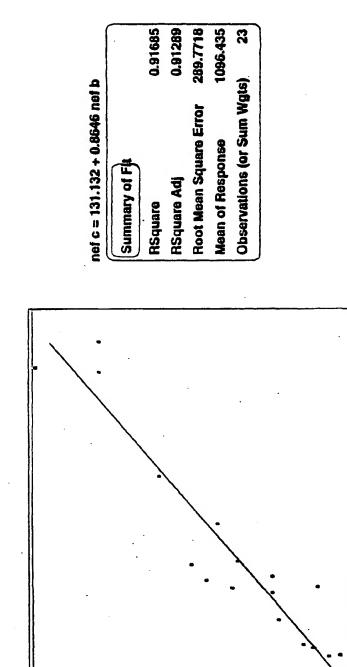


FIGURE 25



nef b

Comparison of Clade B vs. Clade C Anti-nef T Cell

Responses in Clade B HIV-Infected Subjects

3500

3000

2500

o ten 1500

1000

- 009

2000

MRKAd5pol MER1062 (MRKAd5 Pre-Adenoviral Vector Containing the IA opt pol Coding Region)

1				GAAGCCAATA	
	GTAGTAGTTA	TTATATGGAA	TAAAACCTAA	CTTCGGTTAT	ACTATTACTC
51	GGGGTGGAGT				
	CCCCACCTCA	AACACTGCAC	CGCGCCCCGC	ACCCTTGCCC	CGCCCACTGC
101				GTGTGGCGGA	
	ATCATCACAC	CGCCTTCACA	CTACAACGTT	CACACCGCCT	TGTGTACATT
151				GTGTGCGCCG	
	CGCTGCCTAC	ACCGTTTTCA	CTGCAAAAAC	CACACGCGGC	CACATGTGTC
201			•	GATGTTGTAG	
	CTTCACTGTT	AAAAGCGCGC	CAAAATCCGC	CTACAACATC	ATTTAAACCC
251	CGTAACCGAG				
	GCATTGGCTC	ATTCTAAACC	GGTAAAAGCG	CCCTTTTGAC	TTATTCTCCT
301				TAGCGCGTAA	
	TCACTTTAGA	CTTATTAAAA	CACAATGAGT	ATCGCGCATT	ATAAACAGAT
351				AGACTCGCCC	
	CCCGGCGCCC	CTGAAACTGG	CAAATGCACC	TCTGAGCGGG	TCCACAAAAA
401				TTGGCGTTTT	
	GAGTCCACAA	AAGGCGCAAG	GCCCAGTTTC	AACCGCAAAA	TAATAATATC
451				CATATCATAA	
	CGCCGGCGCT	AGGTAACGTA	TGCAACATAG	GTATAGTATT	ATACATGTAA
501				TGTTGACATT	
	ATATAACCGA	GTACAGGTTG	TAATGGCGGT	ACAACTGTAA	CTAATAACTG
551				ATTAGTTCAT	
	ATCAATAATT	ATCATTAGTT	AATGCCCCAG	TAATCAAGTA	TCGGGTATAT
601				ATGGCCCGCC	
	ACCTCAAGGC	GCAATGTATT	GAATGCCATT	TACCGGGCGG	ACCGACTGGC
651				ATGACGTATG	
	GGGTTGCTGG	GGGCGGGTAA	CTGCAGTTAT	TACTGCATAC	AAGGGTATCA
	AACGCCAATA				
	TTGCGGTTAT	CCCTGAAAGG	TAACTGCAGT	TACCCACCTC	ATAAATGCCA
751		CTTGGCAGTA	CATCAAGTGT	ATCATATGCC	AAGTACGCCC
	TTTGACGGGT	GAACCGTCAT	GTAGTTCACA	TAGTATACGG	TTCATGCGGG
801				GCCTGGCATT	
	GGATAACTGC	AGTTACTGCC	ATTTACCGGG	CGGACCGTAA	TACGGGTCAT
851	CATGACCTTA	TGGGACTTTC	CTACTTGGCA	GTACATCTAC	GTATTAGTCA
				CATGTAGATG	

7 i jure 26A

901	TCGCTATTAC AGCGATAATG	GTACCACTAC	CGGTTTTGGC GCCAAAACCG	AGTACATCAA TCATGTAGTT	TGGGCC EA ACCCGCACCT
95 <u>1</u>	TAGCGGTTTG ATCGCCAAAC	ACTCACGGGG TGAGTGCCCC	ATTTCCAAGT TAAAGGTTCA	CTCCACCCCA GAGGTGGGGT	TTGACGTCAA AACTGCAGTT
1001	TGGGAGTTTG ACCCTCAAAC	TTTTGGCACC AAAACCGTGG	AAAATCAACG TTTTAGTTGC	GGACTTTCCA CCTGAAAGGT	AAATGTCGTA TTTACAGCAT
1051	TGTTGAGGCG	GGGTAACTGC	GTTTACCCGC	GTAGGCGTGT CATCCGCACA	TGCCACCCTC
1101		CGTCTCGAGC	AAATCACTTG	GCAGTCTAGC	GGACCTCTGC
1151	GGTAGGTGCG	ACAAAACTGG	AGGTATCTTC	ACACCGGGAC TGTGGCCCTG	GCTAGGTCGG
1201	AGGCGCCGGC	CCTTGCCACG	TAACCTTGCG	GGATTCCCCG CCTAAGGGGC	ACGGTTCTCA
1251	CTCTAGATGG	TACCGGGGGT	AGAGGGGGTA	TGAGACTGTG ACTCTGACAC	GGACACTTCG
1301	ACTTCGGACC	GTACCTACCG	GGGTTCCACT	AGCAGTGGCC TCGTCACCGG	GGACTGACTC
1351	CTCTTCTAGT	TCCGGGACCA	CCTTTAGACG	ACTGAGATGG TGACTCTACC	TCTTCCTCCC
1401	GTTTTAGAGG	TTCTAACCGG	GGCTCTTGGG	CTACAACACC GATGTTGTGG	GGACACAAAC
1451	GGTAGTTCTT	CTTCCTGAGG	TGGTTCACCI	CCTTCGACCA	GGACTTCAGG CCTGAAGTCC
1501	CTCGACTTGT	TCTCCTGGGI	CCTGAAGACC	CTCCACGTCG	TGGGCATCCC ACCCGTAGGG
1551	GGTGGGGCGA	CCGGACTTCI	TCTTCTTCAG	ACACTGACAC	CTGGCTGTGG
•	CCCTACGGAT	GAAGAGACAC	GGGGACCTAC	TCCTGAAGIC	GAAGTACACT CTTCATGTGA
1651	CGGAAGTGG1	AGGGGAGGT	GTTGTTACTC	TGGGGACCGT	TCAGGTACCA AGTCCATGGT
	CATGTTACAC	GACGGGGTC	CGACCTTCCC	GAGGGGACGG	: ATCTTCCAGT : TAGAAGGTCA
	GGAGGTACT	GTTCTAGGAC	CTCGGGAAGT	CCTTCGTCT1	CCCTGACATT GGGACTGTAA
1801	CACTAGATG	AGTACATGG CONTRACT :	TGCCCTGTAT	r Gregecicio A Caccegagao	ACCTGGAGAT TGGACCTCTA



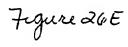
1851		A CCAAGA TCCTGGTTCT		
1901	• • • • • • • • • • • • • • • • • • • •	CACCCCTGAC GTGGGGACTG		
1951		ATGAGCTGCA TACTCGACGT		
2001		AAGGACTCCT TTCCTGAGGA		
2051		CTGGGCCTCC GACCCGGAGG		
2101		TGCTGAGGGG ACGACTCCCC		
2151		GCTGAGCTGG CGACTCGACC	 	
2201		TGGGGTGTAC ACCCCACATG		
2251		AGGGCCAGGG TCCCGGTCCC	 	
2301		CTGAAGACTG GACTTCTGAC		
2351		GAAGCAGCTG CTTCGTCGAC		
2401	TCCATTGTGA AGGTAACACT	TCTGGGGCAA AGACCCCGTT	 	
2451		GAGACCTGGT CTCTGGACCA	 	
2501		GTTTGTGAAC CAAACACTTG		
2551		AGCCCATTGT TCGGGTAACA	 	
2601		GAGACCAAGC CTCTGGTTCG		ACCAACAGGG TGGTTGTCCC
2651	GCAGGCAGAA CGTCCGTCTT			GAAGACTGCC CTTCTGACGG
2701				AGGTGAACAT TCCACTTGTA
2751	TGTGACTGCC ACACTGACGG			CAGCCTGATC GTCGGACTAG

Figure 26 C

2801	AGTCTGAGTC	T CTGGTG	AACCAGATCA	TTGAGCAGCT	GATCAA
	TCAGACTCAG	ACTCGACCAC	TTGGTCTAGT	AACTCGTCGA	CTAGTTCTTC
2851	GAGAAGGTGT	ACCTGGCCTG	GGTGCCTGCC	CACAAGGGCA	TTGGGGGCAA
	CTCTTCCACA	TGGACCGGAC	CCACGGACGG	GTGTTCCCGT	AACCCCCGTT
2901	TGAGCAGGTG	GACAAGCTGG	TGTCTGCTGG	CATCAGGAAG	GTGCTGTTCC
	ACTCGTCCAC	CTGTTCGACC	ACAGACGACC	GTAGTCCTTC	CACGACAAGG
2951	TGGATGGCAT ACCTACCGTA	TGACAAGGCC ACTGTTCCGG	CAGGATGAGC GTCCTACTCG	ATGAGAAGTA TACTCTTCAT	CCACTCCAAC GGTGAGGTTG
3001		TGGCCTCTGA ACCGGAGACT			
3051		TCCTGTGACA AGGACACTGT			GCCATGCATG CGGTACGTAC
3101	GGCAGGTGGA	CTGCTCCCCT	GGCATCTGGC	AGCTGGCCTG	CACCCACCTG
	CCGTCCACCT	GACGAGGGGA	CCGTAGACCG	TCGACCGGAC	GTGGGTGGAC
3151	GAGGGCAAGG CTCCCGTTCC				GCTACATTGA CGATGTAACT
3201					TACTTCCTGC ATGAAGGACG
3251					TGCCAATGGC ACGGTTACCG
3301	TCCAACTTCA	CTGGGGCCAC	AGTGAGGGCT	GCCTGCTGGT	GGGCTGGCAT
	AGGTTGAAGT	GACCCCGGTG	TCACTCCCGA	CGGACGACCA	CCCGACCGTA
3351					GGGGTGGTGG CCCCACCACC
3401	CCTCCATGAA	CAAGGAGCTG	AAGAAGATCA	TTGGGCAGGT	GAGGGACCAG
	GGAGGTACTT	GTTCCTCGAC	TTCTTCTAGT	AACCCGTCCA	CTCCCTGGTC
3451					TCCACAACTT AGGTGTTGAA
3501	CAAGAGGAAG	GGGGGCATCG	GGGGCTACTC	CGCTGGGGAG	AGGATTGTGG
	GTTCTCCTTC	CCCCCGTAGC	CCCCGATGAG	GCGACCCCTC	TCCTAACACC
3551	ACATCATTGC	CACAGACATC	CAGACCAAGG	AGCTCCAGAA	GCAGATCACC
	TGTAGTAACG	GTGTCTGTAG	GTCTGGTTCC	TCGAGGTCTT	CGTCTAGTGG
3601	AAGATCCAGA	ACTTCAGGGT	GTACTACAGG	GACTCCAGGA	ACCCCCTGTG
	TTCTAGGTCT	TGAAGTCCCA	CATGATGTCC	CTGAGGTCCT	TGGGGGACAC
3651	GAAGGGCCCT	GCCAAGCTGC	TGTGGAAGGG	GGAGGGGGCT	GTGGTGATCC
	CTTCCCGGGA	CGGTTCGACG	ACACCTTCCC	CCTCCCCGA	CACCACTAGG
3701	AGGACAACTC TCCTGTTGAG	TGACATCAAG ACTGTAGTTC	GTGGTGCCCA CACCACGGGT	GGAGGAAGGC	CAAGATCATC GTTCTAGTAG

Figure 26 D

3751	AGGGACTATG TCCCTGATAC	AGCAGAT CO.TCGTCTA	GGCTGGGGAT CCGACCCCTA	GACTGTGTĞĞ CTGACACACC	CCTCCA TA GGAGGT GT
38,01				TGTGCCTTCT ACACGGAAGA	
3851				CCTTGACCCT GGAACTGGGA	
3901				GAAATTGCAT CTTTAACGTA	
3951				GGTGGGGCAG CCACCCGTC	
4001				CTGGGGATGC GACCCCTACG	
4051				GTGGGCGTGG CACCCGCACC	
4101	GGAAAGAATA CCTTTCTTAT	TATAAGGTGG ATATTCCACC	GGGTCTTATG CCCAGAATAC	TAGTTTTGTA ATCAAAACAT	TCTGTTTTGC AGACAAAACG
4151				GTTTGATGGA CAAACTACCT	
4201				GGGCCGGGGT CCCGGCCCCA	
4251				GTCCTGCCCG CAGGACGGGC	CAAACTCTAC GTTTGAGATG .
4301				GCCGTTGGAG CGGCAACCTC	
4351				CCCGCGGGAT GGGCGCCCTA	
4401				GCAGCTTCCC CGTCGAAGGG	
4451				ACAATTGGAT TGTTAACCTA	
4501	GGGAACTTAA CCCTTGAATT	TGTCGTTTCT ACAGCAAAGA	CAGCAGCTGT GTCGTCGACA	TGGATCTGCG ACCTAGACGC	CCAGCAGGTT GGTCGTCCAA
4551	TCTGCCCTGA AGACGGGACT	AGGCTTCCTC TCCGAAGGAG	CCCTCCCAAT GGGAGGGTTA	GCGGTTTAAA CGCCAAATTT	ACATAAATAA TGTATTTATT
4601	AAAACCAGAC TTTTGGTCTG	TCTGTTTGGA AGACAAACCT	TTTGGATCAA AAACCTAGTT	GCAAGTGTCT CGTTCACAGA	TGCTGTCTTT ACGACAGAAA
					GTCTCGGTCG CAGAGCCAGC



4701	TTGAGGGTCC AACTCCCAGG	TCTCTATTTT ATAAAA	TTCCAGGACG AAGGTCCTGC	TGGTAAAGGT ACCATTTCCA	CTGAGA A
4751				GGGGTGGAGG CCCCACCTCC	
4801				AGATGATCCA TCTACTAGGT	
4851				TTCAGTAGCA AAGTCATCGT	
4901				AAAGCGGTTA TTTCGCCAAT	
4951				TGGACTGTAT ACCTGACATA	
5001				TTCATGTTGT AAGTACAACA	
5051				TTTGTCATGT AAACAGTACA	
5101				TGTGACCTCC ACACTGGAGG	
5151	ATGCATTCGT TACGTAAGCA	CCATAATGAT GGTATTACTA	GGCAATGGGC CCGTTACCCG	CCACGGGCGG GGTGCCCGCC	CGGCCTGGGC GCCGGACCCG
5201				GTTGTGTTCC CAACACAAGG	
5251				GGAGGGTGCC CCTCCCACGG	
5301				TTACCCTCAC AATGGGAGTG	
5351				CATGTCTACC GTACAGATGG	
5401	TGAAGAAAAC ACTTCTTTTG	GGTTTCCGGG CCAAAGGCCC	GTAGGGGAGA CATCCCCTCT	TCAGCTGGGA AGTCGACCCT	AGAAAGCAGG TCTTTCGTCC
5451					AAATCACACC TTTAGTGTGG
5501	TATTACCGGC ATAATGGCCG	TGCAACTGGT ACGTTGACCA	AGTTAAGAGA TCAATTCTCT	GCTGCAGCTG CGACGTCGAC	CCGTCATCCC GGCAGTAGGG
5551	TGAGCAGGGG ACTCGTCCCC	GGCCACTTCG CCGGTGAAGC	TTAAGCATGT AATTCGTACA	CCCTGACTCG GGGACTGAGC	CATGTTTTCC GTACAAAAGG
5601	CTGACCAAAT GACTGGTTTA	CCGCCAGAAG GGCGGTCTTC	GCGCTCGCCG CGCGAGCGGC	CCCAGCGATA GGGTCGCTAT	GCAGTTCTTG CGTCAAGAAC

Figure 26F

5651	CAAGGAAGCA GTTCCTTCGT	 	ACCGTCCGCC TGGCAGGCGG	
5701	_		GGTCCCACAG CCAGGGTGTC	
5751		 	CCTCGTTTCG GGAGCAAAGC	
5801		 	TCGTCCAGAC AGCAGGTCTG	
	CATGTCTTTC GTACAGAAAG	 		
5901			CCAGGGTGCG GGTCCCACGC	
5951		 	TCGCCCTGCG AGCGGGACGC	
6001		 	CCCCTCCGCG GGGGAGGCGC	
6051			CGCACGAGGG GCGTGCTCCC	
6101		 	AATACCGATT TTATGGCTAA	
6151		 	CTCGCATTCC GAGCGTAAGG	
6201			GGTTTCCCCC CCAAAGGGGG	
6251		 	CGGTGTCCAC GCCACAGGTG	
6301		 	CTTGAGAGGC GAACTCTCCG	CTGTCCTCGA GACAGGAGCT
6351		 	ACTCGGACCA TGAGCCTGGT	CTCTGAGACA GAGACTCTGT
6401	AAGGCTCGCG TTCCGAGCGC			AGGGGTAGCG TCCCCATCGC
6451	GTCGTTGTCC CAGCAACAGG			AGACACATGT TCTGTGTACA
6501	CGCCCTCTTC GCGGGAGAAG			GTAGGCCACG CATCCGGTGC
6551	TGACCGGGTG ACTGGCCCAC			GGGCGCGTTC CCCGCGCAAG

Figure 266

6601	GTCCTCACTC	TCTTCCGCAT	CGCTGTCTGC	GAGGGCCAGG	TEPTOLEERG
	CAGGAGTGAG I	AGGCGTA	GCGACAGACG	CTCCCGGTCG	ACAACO
6651	AGTACTCCCT (TCATGAGGGA (CTGAAAAGCG	GGCATGACTT	CTGCGCTAAG	ATTGTCAGTT TAACAGTCAA
6701	TCCAAAAACG AGGTTTTTGC	AGGAGGATTT TCCTCCTAAA	GATATTCACC CTATAAGTGG	ACCGGGCGCC	TGATGCCTTT ACTACGGAAA
6751	GAGGGTGGCC CTCCCACCGG	GCATCCATCT CGTAGGTAGA	CCAGTCTTTT	CTGTTAGAAA	AACAACAGTT
CD01	GCTTGGTGGC	** > CC > CC C	тасассесст	TICGACAGCAA	CTTGGCGATG
6801	CGAACCACCG	TTTGCTGGGC	ATCTCCCGCA	ACCTGTCGTT	GAACCGCTAC
6851	GAGCGCAGGG	TTTGGTTTTT	GTCGCGATCG	GCGCGCTCCT	TGGCCGCGAT
	CTCGCGTCCC	AAACCAAAAA	CAGCGCTAGC	CGCGCGAGGA	ACCGGCGCTA
6901	GTTTAGCTGC	ACGTATTCGC	GCGCAACGCA	CCGCCATTCG	GGAAAGACGG
	CAAATCGACG	TGCATAAGCG	CGCGTTGCGT	GGCGGTAAGC	CCTTTCTGCC
6951	TGGTGCGCTC	GTCGGGCACC	AGGTGCACGC	GCCAACCGCG	GTTGTGCAGG
		•		CGGTTGGCGC	
7001	GTGACAAGGT	CAACGCTGGT	GGCTACCTCT	CCGCGTAGGC	GCTCGTTGGT
	CACTGTTCCA	GTTGCGACCA	CCGATGGAGA	GGCGCATCCG	CGAGCAACCA
7051	CCAGCAGAGG	CGGCCGCCCT	TGCGCGAGCA	GAATGGCGGT	AGGGGGTCTA
	GGTCGTCTCC	GCCGGCGGGA	ACGCGCTCGT	CTTACCGCCA	TCCCCCAGAT
7101	GCTGCGTCTC	GTCCGGGGGG	TCTGCGTCCA	CGGTAAAGAC	CCCGGGCAGC
	CGACGCAGAG	CAGGCCCCCC	AGACGCAGGT	GCCATTTCTG	GGGCCCGTCG
7151	AGGCGCGCGT	CGAAGTAGTC	TATCTTGCAT	CCTTGCAAGT	CTAGCGCCTG
	TCCGCGCGCA	GCTTCATCAG	ATAGAACGTA	GGAACGTTCA	GATCGCGGAC
7201	CTGCCATGCG	CGGGCGGCAA	GCGCGCGCTC	GTATGGGTTG	AGTGGGGGAC
	GACGGTACGC	GCCCGCCGTT	CGCGCGCGAG	CATACCCAAC	TCACCCCTG
7251	CCCATGGCAT	GGGGTGGGTG	AGCGCGGAGG	CGTACATGCC	GCAAATGTCG
	GGGTACCGTA	CCCCACCCAC	TCGCGCCTCC	GCATGTACGG	CGTTTACAGC
7301	TAAACGTAGA	GGGGCTCTCT	GAGTATTCC	AGATATGTAG	GGTAGCATCT
	ATTTGCATCT	CCCCGAGAGA	CTCATAAGGT	TCTATACATC	CCATCGTAGA
7351	TCCACCGCGG	ATGCTGGCGC	GCACGTAAT	GTATAGTTCG	TCCGAGGGAG
	AGGTGGCGCC	TACGACCGCG	CGTGCATTAC	CATATCAAGC	ACCCTCCCTC
7401	CGAGGAGGTC	GGGACCGAGG	TTGCTACGG	CGGGCTGCTC	TGCTCGGAAG
	GCTCCTCCAG	CCCTGGCTCC	AACGATGCCC	GCCCGACGAG	ACGAGCCTTC
7451	ACTATCTGCC	TGAAGATGGC	ATGTGAGTT	GATGATATGG	TTGGACGCTG
	TGATAGACGG	ACTTCTACCO	TACACTCAA	CTACTATACO	AACCTGCGAC
7501	GAAGACGTTG	AAGCTGGCGT	CTGTGAGAC	TACCGCGTCA	CGCACGAAGG
	CTTCTGCAAC	TTCGACCGC	GACACTCTG	ATGGCGCAGT	GCGTGCTTCC

Figure 26 H

7551	AGGCGTAGGA	GCCAGC	TTGTTGACCA	GCTCGGCGGT	GACCTG
	TOUGOATOOT	CAGCGCGTCG	AACAACTCCT	CCACCCCCCA	CTGGACGTGC
			MCMC1001	Canoccoccii	C10@1010C
7601	TCTAGGGCGC	AGTAGTCCAG	GGTTTCCTTG	ATGATGTCAT	ACTTATCCTG
	AGATCCCGCG	TCATCAGGTC	CCAAAGGAAC	TACTACAGTA	TGAATAGGAC
7651	MCCCHAIMMAININ	TTCCACAGCT	CCCCCmmcxc	CACAAACMCE	mcccccmcmm
7031					
	AGGGAAAAA	AAGGTGTCGA	GCGCCAACTC	CTGTTTGAGA	AGCGCCAGAA
7701	TCCAGTACTC	TTGGATCGGA	AACCCGTCGG	CCTCCGAACG	GTARGAGCCT
		AACCTAGCCT			
	AGGICAIGAG	MACCIMOCCI	TIGGGCMGCC	GGAGGCTTGC	CATTLICGGA
				•	
7751	AGCATGTAGA	ACTGGTTGAC	GGCCTGGTAG	GCGCAGCATC	·CCTTTTCTAC
	TCGTACATCT	TGACCAACTG	CCGGACCATC	CGCGTCGTAG	GGAAAAGATG
			••••		
2001	000000000	T1			magama, aac
7801		TATGCCTGCG			
	CCCATCGCGC	ATACGGACGC	GCCGGAAGGC	CTCGCTCCAC	ACCCACTCGC
					•
7851	СРУУССАСАС	CCTGACCATG	ACTITICACCT	አ ርጥርርጥልጥጥጥ	CAACTCACTC
, , , ,					
	GTTTCCACAG	GGACTGGTAC	TGAAACTCCA	TGACCATAAA	CTTCAGTCAC
7901	TCGTCGCATC	CGCCCTGCTC	CCAGAGCAAA	AAGTCCGTGC	GCTTTTTGGA
	ACCACCCTAC	GCGGGACGAG	ここかしかいこかかか	TTCAGGCACG	CCAAAAACCT
		ocooon.com	0010100111	1101000100	COLUMNICO
7951		GGCAGGGCGA			
	TGCGCCTAAA	CCGTCCCGCT	TCCACTGTAG	CAACTTCTCA	TAGAAAGGGC
8001	CCCCACCCAT	AAAGTTGCGT	בתיכאתיכיכיכא	ACCOMOCOCO	CACCOCCAA
0001			•		
	GCGCTCCGTA	TTTCAACGCA	CACTACGCCT	TCCCAGGGCC	GTGGAGCCTT
8051	CGGTTGTTAA	TTACCTGGGC	GGCGAGCACG	ATCTCGTCAA	AGCCGTTGAT
		AATGGACCCG			
	GCCAACAAII	NY 1 GOVECEG	CCGC1CG1GC	INGNOCAGII	ICGGCAACIA
8101	GTTGTGGCCC	ACAATGTAAA	GTTCCAAGAA	GCGCGGGATG	CCCTTGATGG
	CAACACCGGG	TGTTACATTT	CAAGGTTCTT	CGCGCCCTAC	GGGAACTACC
8151	3300033mmm	TTTAAGTTCC	mooma domea	000000000000000000000000000000000000000	0020000200
9131					
	TTCCGTTAAA	AAATTCAAGG	AGCATCCACT	CGAGAAGTCC	CCTCGACTCG
8201	CCGTGCTCTG	AAAGGGCCCA	GTCTGCAAGA	TGAGGGTTGG	AAGCGACGAA
		TTTCCCGGGT			
	GGCACGAGAC	111111111111111111111111111111111111111	CAGACGITCI	ACICCAACC	1106016011
8251	TGAGCTCCAC	AGGTCACGGG	CCATTAGCAT	TTGCAGGTGG	TCGCGAAAGG
	ACTCGAGGTG	TCCAGTGCCC	GGTAATCGTA	AACGTCCACC	AGCGCTTTCC
0001					
8301					GCAGTAGAAG
	AGGATTTGAC	CGCTGGATAC	CGGTAAAAAA	GACCCCACTA	CGTCATCTTC
8351	GTAAGCGGGT	CUNTRACCCA	CCCCTCCCAT	CCAACCTTCC	CCCCTACCTC
0001					
	LATTUGUUCA	CANCARGGGT.	COLLAGGGTA	GGTTCCAAGC	GCCGATCCAG
8401	TCGCGCGCA	GTCACTAGAG	GCTCATCTCC	GCCGAACTTC	ATGACCAGCA
					TACTGGTCGT
				001 marr 1 65	
					ATAGGTCTCT
	ACTTCCCGTG	CTCGACGAAG	GGTTTCCGGG	GGTAGGTTCA	TATCCAGAGA

Figure 26I

8501	ACATCGTAGG TGTAGCATCC	TAAAGAG ACTGTTTCTC	ACGCTCGGTG TGCGAGCCAC	CGAGGATGCG GCTCCTACGC	AGCCGA GGG TCGGCTAGCC
8551	GAAGAACTGG CTTCTTGACC	ATCTCCCGCC TAGAGGGCGG	ACCAATTGGA TGGTTAACCT	GGAGTGGCTA CCTCACCGAT	TTGATGTGGT AACTACACCA
8601	GAAAGTAGAA CTTTCATCTT	GTCCCTGCGA CAGGGACGCT	CGGGCCGAAC GCCCGGCTTG	ACTCGTGCTG TGAGCACGAC	GCTTTTGTAA CGAAAACATT
8651	TTTGCACGCG	AGTACTGGCA TCATGACCGT	CGCCACGTGC	CCGACATGTA	GGACGTGCTC
8701	CAACTGGACT	CGACCGCGCA GCTGGCGCGT	GTTCCTTCGT	CTCACCCTTA	AACTCGGGGA
8751	GCGGACCGCC	GTTTGGCTGG CAAACCGACC	ACCAGAAGAT	GAAGCCGACG	AACAGGAACT
8801	GGCAGACCGA	GCTCGAGGGG CGAGCTCCCC	TCAATGCCAC	CTAGCCTGGT	GGTGCGGCGC
8851	GCTCGGGTTT	GTCCAGATGT CAGGTCTACA	GGCGCGCCC	GCCAGCCTCG	AACTACTGTT
8901	GTAGCGCGTC	ATGGGAGCTG TACCCTCGAC	AGGTACCAGA	CCTCGAGGGC	GCCGCAGTCC
8951	AGTCCGCCCT	CGAGGACGTC	CAAATGGAGC	GTATCTGCCC	
9001	CCGATCTAGG	TCCACTATGG	ATTAAAGGTC	CCCGACCAAC	GTGGCGGCGT CACCGCCGCA
9051	GCTACCGAAC	GTTCTCCGGC	GTAGGGGCGC	CGCGCTGATG	GGTACCGCGC CCATGGCGCG
9101	CCGCCCGCCA	CCCGGCGCCC	CCACAGGAAC	: CTACTACGTA	CTAAAAGCGG GATTTTCGCC
9151	ACTGCGCCCG	CTCGGGGGCC	TCCATCCCCC	CCGAGGCCTG	CCGCCGGGAG GGCGGCCCTC
9201	TCCCCCGTCC	CCGTGCAGCC	ececcecece	CCCGTCCTCG	TGGTGCTGCG ACCACGACGC
	GCGCATCCAZ	CGACCGCTTC	CGCTGCTGC	CCGCCAACTA	CTCCTGAATC GAGGACTTAG
	ACCGCGGAGA	CGCACTTCTC	CIGCCCGGG	CACTCGAACT	A ACCTGAAAGA TGGACTTTCT
	CTCAAGCTGT	CTTAGTTAAA	A GCCACAGCA	A CTGCCGCCGC	TGGCGCAAAA ACCGCGTTTT
9401	TCTCCTGCAC AGAGGACGTC	CAGAGGACTY	TTGTCTTGA	A TCCGCTAGA	GGCCATGAAC GCCGGTACTTG

Figure 26 J

9451	TGCTCGATCT	CTCCTG	GAGATCTCCG	CGTCCGGCTC	GCTCCA T
		GAAGGAGGAC			
	ACCROC SISC.	anoanoano	CICIMONOC	0030000030	caroo16ccr
9501		TCGTTGGAAA	mcccccc a m	CACCMCCCAC	*************
3201					
	CCGCCGCTCC	AGCAACCTTT	ACGCCCGGTA	CTCGACGCTC	TTCCGCAACT
9551	GGCCTCCCTC	GTTCCAGACG	CGGCTGTAGA	CCACGCCCCC	TTCGGCATCG
	CCGGAGGGAG	CAAGGTCTGC	GCCGACATCT	GCTGCGGGG	AAGCCGTAGC
9601	CGGGCGCGCA	TGACCACCTG	CGCGAGATTG	AGCTCCACGT	GCCGGGCGAA
	GCCCGCGCGT	ACTGGTGGAC	GCGCTCTAAC	TCGAGGTGCA	CGGCCCGCTT
					•
9651	GACGGCGTAG	TTTCGCAGGC	CCTCAAAGAG	CTACTTCACC	CTCCTCCCCG
		AAAGCGTCCG			
	CIGCOGGIC		CGACIIICIC	Chichheice	CACCACCGCC
9701	manamana	CACGAAGAAG	m>0>m>3000	3.000m0003.3	CCDCCADOCC .
9/01	_				
	ACACAAGACG	GTGCTTCTTC	ATGTATTGGG	TCGCAGCGTT	GCACCTAAGC
9751	TTGATATCCC				
	AACTATAGGG	GGTTCCGGAG	TTCCGCGAGG	TACCGGAGCA	TCTTCAGGTG
		·			
9801	GGCGAAGTTG	AAAAACTGGG	AGTTGCGCGC	CGACACGGTT	AACTCCTCCT
	CCGCTTCAAC	TTTTTGACCC	TCAACGCGCG	GCTGTGCCAA	TTGAGGAGGA
9851	CCAGAAGACG	GATGAGCTCG	GCGACAGTGT	CGCGCACCTC	GCGCTCAAAG
•	GGTCTTCTGC	CTACTCGAGC	CCCTCTCACA	GCGCGTGGAG	CGCGAGTTTC
9901	GCTACAGGGG	CCTCTTCTTC	ተጥር ተጥር ል ነርር	תרכתרתתרר א	ጥ ል ልርርርርርርርር
,,,,,		GGAGAAGAAG			
	CGNIGICCCC	Garamana	ANGANGIING	NOCACANGG1	ATTCCCGGAG
9951	0000000000	memmemeeee	cacamacaaa	10000000	COCCCCCC
9951		TCTTCTGGCG			
	GGGAAGAAGA	AGAAGAUCGU	CGCCACCCCC	Tececerer	GCCGCCGCTG
10001		CGGGAGGCGG			
	CTGCCGCGTG	GCCCTCCGCC	AGCTGTTTCG	CGAGCTAGTA	GAGGGGCGCC
					• :
10051	CGACGGCGCA	TGGTCTCGGT	GACGGCGCGG	CCGTTCTCGC	GGGGGCGCAG
	GCTGCCGCGT	ACCAGAGCCA	CTGCCGCGCC	GGCAAGAGCG	CCCCCGCGTC
10101	TTGGAAGACG	CCGCCCGTCA	TGTCCCGGTT	ATGGGTTGGC	GGGGGCTGC
	AACCTTCTGC	GGCGGGCAGT	ACAGGGCCAA	TACCCAACCG	CCCCCGACG
		00000001			
10151	ראזייכרפכראפ	GGATACGGCG	СТАВССВТСС	አጥርጥር አ ልሮ ልል	ጥጥርጥጥርጥርጥል
10101		CCTATGCCGC			
	GIACGCCGIC	CCIAIGCCGC	GNIIGCIACG	INGNGIIGII	ANCANCACAI
		0000010001	00000000000	maaaaa maaa	CCGGATCGGA
T070T					
	CCATGAGGCG	GCGGCTCCCT	GGACTCGCTC	AGGCGTAGCT	GGCCTAGCCT
10251					GGTAGGCTGA
	TTTGGAGAGC	TCTTTCCGCA	GATTGGTCAG	TGTCAGCGTT	CCATCCGACT
10301	GCACCGTGGC	GGGCGGCAGC	GGGCGGCGGT	CGGGGTTGTT	TCTGGCGGAG
	CGTGGCACCG	CCCGCCGTCG	CCCGCCGCCA	GCCCCAACAA	AGACCGCCTC
10351	GTGCTGCTGA	TGATGTAATT	AAAGTAGGCG	GTCTTGAGAC	GGCGGATGGT
					CCGCCTACCA

Figure 26 K

10401	CGACAGAAGC GCTGTCTTCG	A TGTCCT TACAGGA	TGGGTCCGGC ACCCAGGCCG	CTGCTGAATG GACGACTTAC	CCCAGG T GCGTCC A
10451	CGGCCATGCC GCCGGTACGG	CCAGGCTTCG GGTCCGAAGC			
10501		GCCTTTCTAC CGGAAAGATG			
10551		GCATCTATCG CGTAGATAGC			
10601	GGCGCCCTCT	TCCTCCCATG AGGAGGGTAC			
10651	AGCAGGGCTA TCGTCCCGAT	GGTCGGCGAC CCAGCCGCTG	AACGCGCTCG TTGCGCGAGC	GCTAATATGG CGATTATACC	CCTGCTGCAC GGACGACGTG
10701	CTGCGTGAGG	GTAGACTGGA CATCTGACCT	AGTCATCCAT	GTCCACAAAG	CGGTGGTATG
10751	CGCCCGTGTT	GATGGTGTAA CTACCACATT	GTGCAGTTGG	CCATAACGGA	CCAGTTAACG
10801	GTCTGGTGAC	CCGGCTGCGA	GAGCTCGGTG	TACCTGAGAC	GCGAGTAAGC
10851	CCTCGAGTCA	GGCCGACGCT	CGTTGCAAGT	CCGCACCAGG	TACTGGTATC
		TTATGCATCA		•	
10901	GGTGGTTTTT	CACGCCGCCG	CCGACCGCCA	TCTCCCCGGT	CGCATCCCAC
10951	GCCGGGGCTC	CGGGGGGGAG	ATCTTCCAAC TAGAAGGTTG	ATAAGGCGAT TATTCCGCTA	GATATCCGTA CTATAGGCAT
11001		GACATCCAGG CTGTAGGTCC			
11051					AAAGTGCTCC TTTCACGAGG
11101	ATGGTCGGGA TACCAGCCCT	CGCTCTGGCC	GGTCAGGCGC CCAGTCCGCG	GCGCAATCGT CGCGTTAGCA	TGACGCTCTA ACTGCGAGAT
11151	GACCGTGCAA CTGGCACGTT	AAGGAGAGCC TTCCTCTCGG	TGTAAGCGGG ACATTCGCCC	CACTCTTCCG	TGGTCTGGTG ACCAGACCAC
11201	GATAAATTCG CTATTTAAGC	CAAGGGTATC	ATGGCGGACG TACCGCCTGC	ACCGGGGTTC TGGCCCCAAG	GAGCCCCGTA CTCGGGGCAT
	TCCGGCCGTC	CGCCGTGATC	CATGCGGTTA	CCCCCCCCC	GTCGAACCCA CAGCTTGGGT
11301	GGTGTGCGAC	GTCAGACAAC	GGGGGAGTGC	TCCTTTTGGC	TTCCTTCCAG AAGGAAGGTC

Figure 26L

	cececcecce			
11401	AAGCGGTTAG TTCGCCAATC		AGTGGCTCGC TCACCGAGCG	
11451			GGGACCCCCG CCCTGGGGGC	
11501			TTGCCTCCCC AACGGAGGGG	
11551		 	GACGAGCCCC CTGCTCGGGG	•••
11601	· · - · · -	 	GCGCCCCCT	
11651		 	GGGCACCCTC CCCGTGGGAG	
11701		 	GACGCGGCAG CTGCGCCGTC	
11751		 	CTACCTGGAC GATGGACCTG	
11801		 	CTCCTGAGCG GAGGACTCGC	
11851		 	TACGTGCCGC ATGCACGGCG	GGCAGAACCT CCGTCTTGGA
11901		 	GGAGATGCGG CCTCTACGCC	GATCGAAAGT CTAGCTTTCA
11951			TGAATCGCGA ACTTAGCGCT	GCGGTTGCTG CGCCAACGAC
12001				GTCCCGCGCG CAGGGCGCGC
12051				CAGACGGTGA GTCTGCCACT
12101				GCGTACGCTT CGCATGCGAA
12151				GGGACTTTGT CCCTGAAACA
12201				GCGCAGCTGT CGCGTCGACA
12251				GGATGCGCTG CCTACGCGAC

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12301	CTAAACATAG GATTTGTATC	T GCCCGA ATLTCGGGCT	GGGCGGCGACC	CTGCTCGATT GACGAGCTAA	TGATAA T ACTATTTGTA
12351	CCTGCAGAGC GGACGTCTCG	ATAGTGGTGC TATCACCACG	AGGAGCGCAG TCCTCGCGTC	CTTGAGCCTG GAACTCGGAC	GCTGACAAGG CGACTGTTCC
12401	TGGCCGCCAT ACCGGCGGTA	CAACTATTCC GTTGATAAGG	ATGCTTAGCC TACGAATCGG	TGGGCAAGTT ACCCGTTCAA	TTACGCCCGC AATGCGGGCG
12451	AAGATATACC TTCTATATGG			GACAAGGAGG CTGTTCCTCC	
12501	GGGGTTCTAC CCCCAAGATG	ATGCGCATGG TACGCGTACC	CGCTGAAGGT GCGACTTCCA	GCTTACCTTG CGAATGGAAC	AGCGACGACC TCGCTGCTGG
12551	TGGGCGTTTA ACCCGCAAAT	TCGCAACGAG AGCGTTGCTC	CGCATCCACA GCGTAGGTGT	AGGCCGTGAG TCCGGCACTC	CGTGAGCCGG GCACTCGGCC
12601	CGGCGCGAGC GCCGCGCTCG	TCAGCGACCG AGTCGCTGGC	CGAGCTGATG GCTCGACTAC	CACAGCCTGC GTGTCGGACG	AAAGGGCCCT TTTCCCGGGA
12651	GGCTGGCACG CCGACCGTGC	GGCAGCGGCG CCGTCGCCGC	ATAGAGAGGC TATCTCTCCG	CGAGTCCTAC GCTCAGGATG	TTTGACGCGG AAACTGCGCC
12701	GCGCTGACCT CGCGACTGGA	GCGCTGGGCC CGCGACCCGG	CCAAGCCGAC	GCCCCTGGA CGCGGGACCT	GGCAGCTGGG CCGTCGACCC
12751	GCCGGACCTG CGGCCTGGAC	GGCTGGCGGT CCGACCGCCA	GGCACCCGCG	CGCGCTGGCA GCGCGACCGT	ACGTCGGCGG TGCAGCCGCC
12801	CGTGGAGGAA GCACCTCCTT	TATGACGAGG ATACTGCTCC	ACGATGAGTA TGCTACTCAT	CGAGCCAGAG	GACGGCGAGT CTGCCGCTCA
12851	ACTAAGCGGT TGATTCGCCA	GATGTTTCTG CTACAAAGAC	ATCAGATGAT TAGTCTACTA	GCAAGACGCA CGTTCTGCGT	ACGGACCCGG TGCCTGGGCC
12901	CGGTGCGGGC GCCACGCCCG	GGCGCTGCAG	AGCCAGCCGT	CCGGCCTTAA	CTCCACGGAC GAGGTGCCTG
12951	GACTGGCGCC CTGACCGCGG	AGGTCATGGA TCCAGTACCI	CCGCATCATG GGCGTAGTAC	TCGCTGACTG AGCGACTGAC	CGCGCAATCC GCGCGTTAGG
13001	ACTGCGCAAG	GCCGTCGTCG	GCGTCCGGTT	GGCCGAGAGG	GCAATTCTGG CGTTAAGACC
13051	AAGCGGTGGT TTCGCCACCA	GGGCGCGCGCG	GCAAACCCCA GCGTTTGGGGT	CGCACGAGAA GCGTGCTCTT	GGTGCTGGCG CCACGACCGC
13101	ATCGTAAACG TAGCATTTGC	CGCTGGCCGA	AAACAGGGCC TTTGTCCCGG	TAGGCCGGGC	ACGAGGCCGG
13151	CCTGGTCTAC GGACCAGATG	GACGCGCTGC CTGCGCGACG	TTCAGCGCGT AAGTCGCGCA	GGCTCGTTAC	AACAGCGGCA TTGTCGCCGT
13201	ACGTGCAGAC TGCACGTCTG	CAACCTGGAC GTTGGACCTC	CGGCTGGTGG CCCGACCAC	GGGATGTGCG CCCTACACGC	CGAGGCCGTG GCTCCGGCAC

Figure 26 N.

13251	GCGCAGCGTG CGCGTCGCAC	A CGCGCGCA TCGCGCGCGT	GCAGCAGGGC CGTCGTCCCG	AACCTGGGCT TTGGACCCGA	CCATGG C GGTACCAACG
13301				CAACGTGCCG GTTGCACGGC	
13351		-		GGCTAATGGT CCGATTACCA	
13401				GACTATTTT CTGATAAAAA	
13451				CCAGGCTTTC GGTCCGAAAG	
13501				GCGACCGCGC CGCTGGCGCG	
13551				CTGCTGCTAA GACGACGATT	
13601				ATACCTAGGT TATGGATCCA	
13651				ATGTGGACGA TACACCTGCT	
13701				GGGCAGGAGG CCCGTCCTCC	
13751				CAACCGGCGG GTTGGCCGCC	
13801				AGCGCATTTT TCGCGTAAAA	
13851				GACGGGGTAA CTGCCCCATT	CGCCCAGCGT GCGGGTCGCA
13901	••••			ACCGGGCATG TGGCCCGTAC	TATGCCTCAA ATACGGAGTT
13,951				ACTTGCATCG TGAACGTAGC	CCCCCCCCC
14001					ACTGGCTACC TGACCGATGG
14051					GGTAACGATG CCATTGCTAC
14101					GCAACCGCAG CGTTGGCGTC
14151					CGCTGCGAAA GCGACGCTTT

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14201	GGAAAGCTTC CCTTTCGAAG	CACCCCAA	GCAGCTTGTC CGTCGAACAG	CGATCTAGGC GCTAGATCCG	CGACGC GCCC
14251	CGCGGTCAGA	TGCTAGTAGC	CCATTTCCAA	GCTTGATAGG	GTCTCTTACC
	GCGCCAGTCT	ACGATCATCG	GGTAAAGGTT	CGAACTATCC	CAGAGAATGG
14301	AGCACTCGCA	CCACCCGCCC	GCGCCTGCTG	GGCGAGGAGG	AGTACCTAAA
	TCGTGAGCGT	GGTGGGCGGG	CGCGGACGAC	CCGCTCCTCC	TCATGGATTT
14351	CAACTCGCTG	CTGCAGCCGC	AGCGCGAAAA	AAACCTGCCT	CCGGCATTTC
	GTTGAGCGAC	GACGTCGGCG	TCGCGCTTTT	TTTGGACGGA	GGCCGTAAAG
14401	CCAACAACGG	GATAGAGAGC	CTAGTGGACA	AGATGAGTAG	ATGGAAGACG
	GGTTGTTGCC	CTATCTCTCG	GATCACCTGT	TCTACTCATC	TACCTTCTGC
14451	TACGCGCAGG ATGCGCGTCC	AGCACAGGGA TCGTGTCCCT	CGTGCCAGGC GCACGGTCCG	CCCCCCCCCC	CCACCCGTCG
14501	TCAAAGGCAC	GACCGTCAGC	GGGGTCTGGT	GTGGGAGGAC	GATGACTCGG
	AGTTTCCGTG	CTGGCAGTCG	CCCCAGACCA	CACCCTCCTG	CTACTGAGCC
14551	CAGACGACAG	CAGCGTCCTG	GATTTGGGAG	GGAGTGGCAA	CCCGTTTGCG
	GTCTGCTGTC	GTCGCAGGAC	CTAAACCCTC	CCTCACCGTT	GGGCAAACGC
14601	CACCTTCGCC	CCAGGCTGGG	GAGAATGTTT	TAAAAAAAAA	AAAAGCATGA
	GTGGAAGCGG	GGTCCGACCC	CTCTTACAAA	TTTTTTTA	TTTTCGTACT
14651	TGCAAAATAA	AAAACTCACC	AAGGCCATGG	CACCGAGCGT	TGGTTTTCTT
	ACGTTTTATT	TTTTGAGTGG	TTCCGGTACC	GTGGCTCGCA	ACCAAAAGAA
14701	GTATTCCCCT CATAAGGGGA	TAGTATGCGG	CGCGCGGCGA	TGTATGAGGA ACATACTCCT	AGGTCCTCCT TCCAGGAGGA
14751	CCCTCCTACG GGGAGGATGC	AGAGTGTGGI TCTCACACCA	GAGCGCGGCGC	CCAGTGGCGG GGTCACCGCC	CGGCGCTGGG
14801	TTCTCCCTTC	GATGCTCCCC	TGGACCCGCC	GTTTGTGCCT	CCGCGGTACC
	AAGAGGGAAG	CTACGAGGGG	ACCTGGGCGG	CAAACACGGA	GGCGCCATGG
14851	TGCGGCCTAC ACGCCGGATG	CGGGGGGAGA	AACAGCATCC TTGTCGTAGG	GTTACTCTGA CAATGAGACT	GTTGGCACCC CAACCGTGGG
14901	CTATTCGACA GATAAGCTGT	CCACCCGTGT	GTACCTGGTG CATGGACCAC	GACAACAAGT CTGTTGTTCA	CAACGGATGT GTTGCCTACA
14951	GGCATCCCTG	AACTACCAGA	A ACGACCACAG	CAACTTTCTC	ACCACGGTCA
	CCGTAGGGAC	TTGATGGTC	TGCTGGTGTC	CTTGAAAGAC	TGGTGCCAGT
15001	TTCAAAACAT AAGTTTTGT	TGACTACAGO ACTGATGTCO	CCGGGGGAGG GGCCCCCCCCC	CAAGCACACACACACACACACACACACACACACACACAC	GACCATCAAT
15051	CTTGACGACO GAACTGCTGO	GGTCGCACT	GGGCGGCGAC CCCCCCGCTG	CTGAAAACCA GACTTTTGG1	A TCCTGCATAC AGGACGTATG
15101	CAACATGCCA	AATGTGAAC	AGTTCATGTT	TACCAATAA	TTTAAGGCGC
	GTTGTACGG	TTACACTTG	CAAGTACAJ	TTATTGGTTA T	AAATTCCGCG

Figure 26 P

					<u></u>
15151	GGGTGATGGT				
	CCCACTACCA	CAGCGCGAAC	GGATGATTCC	TGTTAGTCCA	CCTCGACTTT
		•			
15201	TACGAGTGGG	TGGAGTTCAC	GCTGCCCGAG	GGCAACTACT	CCGAGACCAT
	ATGCTCACCC	ACCTCAAGTG	CGACGGGCTC	CCGTTGATGA	GGCTCTGGTA
15251	GACCATAGAC	CTTATGAACA	ACCCGATCGT	GGAGCACTAC	TTGAAAGTGG
		GAATACTTGT			
	CIGGINICIG	GWHINCIIGI	IGCGCIAGCA	CCICGIGAIG	MCITICACC
4 - 2 4 4	001010101			maaaaam	CTTTTC: C: CC
15301		CGGGGTTCTG	_		
	CGTCTGTCTT	GCCCCAAGAC	CTTTCGCTGT	AGCCCCATTT	CAAACTGTGG
		•			
15351		GACTGGGGTT			
	GCGTTGAAGT	CTGACCCCAA	ACTGGGGCAG	TGACCAGAAC	AGTACGGACC
15401	GGTATATACA	AACGAAGCCT	TCCATCCAGA	CATCATTTTG	CTGCCAGGAT
	CCATATATGT	TTGCTTCGGA	AGGTAGGTCT	GTAGTAAAAC	GACGGTCCTA
15451	GCGGGGTGGA	CTTCACCCAC	AGCCGCCTGA	GCAACTTGTT	GGGCATCCGC
	CGCCCCACCT	GAAGTGGGTG	TCGGCGGACT	CGTTGAACAA	CCCGTAGGCG
15501	AAGCGGCAAC	CCTTCCAGGA	GGGCTTTTAGG	ATCACCTACG	ATGATCTGGA
		GGAAGGTCCT			
	1100000110	00.2.00.001	00000000000		
15551	CCCTCCTAAC	ATTCCCGCAC	יייביייים ביייביי	CCACCCTAC	СРСССРССТ
17331		TAAGGGCGTG		•	
	CCCACCATIG	IANGGGCGIG	MCMMCC INCM.	CCIGCGGAIG	GICCGCICGA
15601	maxxxxx	CACCGAACAG	0000000000		0100110100
15601			• • • • • • • •		
	ACTITCTACT	GTGGCTTGTC	CCGCCCCCAC	CGCGTCCGCC	GICGTIGTCG
15651	-	GCGCGGAAGA			
	TCACCGTCGC	CGCGCCTTCT	CTTGAGGTTG	CGCCGTCGGC	GCCGTTACGT
15701		GACATGAACG			
	CGGCCACCTC	CTGTACTTGC	TAGTACGGTA	AGCGCCGCTG	TGGAAACGGT
15751	CACGGGCTGA	GGAGAAGCGC	GCTGAGGCCG	AAGCAGCGGC	CGAAGCTGCC
	GTGCCCGACT	CCTCTTCGCG	CGACTCCGGC	TTCGTCGCCG	GCTTCGACGG
15801	GCCCCCGCTG	CGCAACCCGA	GGTCGAGAAG	CCTCAGAAGA	AACCGGTGAT
	CGGGGGGCGAC	GCGTTGGGCT	CCACCTCTTC	GGAGTCTTCT	TTGGCCACTA
	0.00.0000				
15851	CAAACCCCTTC	ACAGAGGACA	GCAAGAAAGG	СРСТТРОТО	СТААТААССА
13031		TGTCTCCTGT			
	GIIIGGGGAC	1010100101	CGIICIIIGC	G1G2113113	GATTATICGI
15001	ATGACAGCAC	CONCACCONC	maccccaccm	CCTD CCTTCC	ATTACA ACTTAC
	. TACTGTCGTG				
	. INCIDITEIN	CAMOTOGGIC	ALGOCGICGA	CCMIGONACE	IMIGIIGAIG
15051	00001000	******	COCCESTRO	* CCCCCCCCCCC	CC 3 CIMCCINC 3
TOROT					GCACTCCTGA
	CCGCTGGGAG	TCTGGCCTTA	GGCGAGTACC	TGGGACGAAA	CGTGAGGACT
160,01	CGTAACCTGC				
	GCATTGGACG	CCGAGCCTCG	TCCAGATGAC	CAGCAACGGT	CTGTACTACG
16051					CTTTCCGGTG
	TTCTGGGGCA	CTGGAAGGCG	AGGTGCGCGG	TCTAGTCGTT	GAAAGGCCAC

Figure 26 Q

16101	GTGGGCGCCG	A TGTTGCC	CGTGCACTCC	AAGAGCTTCT	ACAACGA CA
	CACCCGCGGC	T ACAACGG	GCACGTGAGG	TTCTCGAAGA	TGTTGC GT
16151	GGCCGTCTAC	TCCCAACTCA	TCCGCCAGTT	TACCTCTCTG	ACCCACGTGT
	CCGGCAGATG	AGGGTTGAGT	AGGCGGTCAA	ATGGAGAGAC	TGGGTGCACA
16201	TCAATCGCTT	TCCCGAGAAC	CAGATTTTGG	ececeecee	AGCCCCCACC
	AGTTAGCGAA	AGGGCTCTTG	GTCTAAAACC	cececcecc	TCGGGGGTGG
16251	ATCACCACCG	TCAGTGAAAA	CGTTCCTGCT	CTCACAGATC	ACGGGACGCT
	TAGTGGTGGC	AGTCACTTTT	GCAAGGACGA	GAGTGTCTAG	TGCCCTGCGA
16301	ACCGCTGCGC	AACAGCATCG	GAGGAGTCCA	GCGAGTGACC	ATTACTGACG
	TGGCGACGCG	TTGTCGTAGC	CTCCTCAGGT	CGCTCACTGG	TAATGACTGC
16351	CCAGACGCCG	CACCTGCCCC	TACGTTTACA	AGGCCCTGGG	CATAGTCTCG
	GGTCTGCGGC	GTGGACGGGG	ATGCAAATGT	TCCGGGACCC	GTATCAGAGC
16401	CCGCGCGTCC	TATCGAGCCG ATAGCTCGGC	CACTTTTTGA GTGAAAAACT	GCAAGCATGT CGTTCGTACA	CCATCCTTAT GGTAGGAATA
16451	ATCGCCCAGC	AATAACACAG	GCTGGGGCCT	GCGCTTCCCA	AGCAAGATGT
	TAGCGGGTCG	TTATTGTGTC	CGACCCCGGA	CGCGAAGGGT	TCGTTCTACA
16501	TTGGCGGGGC	CAAGAAGCGC	TCCGACCAAC	ACCCAGTGCG	222222222
	AACCGCCCG	GTTCTTCGCG	AGGCTGGTTG	TGGGTCACGC	222222222
16551	CACTACCGCG GTGATGGCGC	CGCCCTGGGG GCGGGACCCC	CGCGCACAAA GCGCGTGTTT	CGCGGCCGCA	CTGGGCGCAC GACCCGCGTG
16601	CACCGTCGAT	GACGCCATCG	ACGCGGTGGT	GGAGGAGGCG	CGCAACTACA
	GTGGCAGCTA	CTGCGGTAGC	TGCGCCACCA	CCTCCTCCGC	GCGTTGATGT
16651	CGCCCACGCC	GCCACCAGTG CGGTGGTCAC	TCCACAGTGG AGGTGTCACC	ACGCGGCCAT TGCGCCGGTA	TCAGACCGTG AGTCTGGCAC
16701	GTGCGCGGAG	CCCGGCGCTA	TGCTAAAATG	AAGAGACGGC	GGAGGCGCGT
	CACGCGCCTC	GGGCCGCGAT	ACGATTTTAC	TTCTCTGCCG	CCTCCGCGCA
16751	AGCACGTCGC TCGTGCAGCG	CACCGCCGCC	GACCCGGCAC CTGGGCCGTG	TGCCGCCCAA	CGCGCGCGC
16801	CGGCCCTGCT GCCGGGACGA	TAACCGCGCA ATTGGCGCGT	CGTCGCACCG	GCCGACGGGC	GGCCATGCGG CCGGTACGCC
16851	GCCGCTCGAA CGGCGAGCTT	GGCTGGCCGC	CCCATAACAG	ACTGTGCCCC TGACACGGGG	CCAGGTCCAG
16901	GCGACGAGCG CGCTGCTCGC	GCCGCCGCAG	CAGCCGCGCGCGCGCGGCGGCGGCGGCGGCGGCGGCGGCG	CATTAGTGCT GTAATCACGA	ATGACTCAGG TACTGAGTCC
16951	GTCGCAGGGG CAGCGTCCCC	CAACGTGTAT GTTGCACATA	TGGGTGCGCG ACCCACGCGC	ACTCGGTTAG TGAGCCAATC	CGGCCTGCGC
17001	GTGCCCGTGC	GCACCGCCC	CCCGCGCAAC	TAGATTGCAA	GAAAAAACTA
	CACGGGCACG	CGTGGGCGGG	GGGCGCGTTG	ATCTAACGTT	CTTTTTTGAT



17051			cceccéccec eccececene	
17101			TGCTCCAGGT ACGAGGTCCA	
17151			 CAGGATTACA GTCCTAATGT	
17201			 TGATGATGAT ACTACTACTA	
17251			 CCAGGCGACG GGTCCGCTGC	
17301			CCCGGCACCA GGGCCGTGGT	
17351		*	 CAAGCGCGTG GTTCGCGCAC	
17401			CCAACGAGCG GGTTGCTCGC	
17451			CTGGCGTTGC GACCGCAACG	
17501			AACACTGCAG TTGTGACGTC	
17551			GCCTAAAGCG CGGATTTCGC	
17601			 CCCAAGCGCC GGGTTCGCGG	
17651			TGGGCTGGAG ACCCGACCTC	
17701			GACTGGGCGT CTGACCCGCA	
17751	*		AGTATTGCCA TCATAACGGT	CCGCCACAGA GGCGGTGTCT
17801	GGGCATGGAG CCCGTACCTC			GCGGATGCCG CGCCTACGGC
17851				GGAGGTGCAA CCTCCACGTT
17901				CGCGCCGTTC GCGCGGCAAG
17951				GCCCTACATC CGGGATGTAG

Figure 265

18001	CTTCCATTGC GAAGGTAACG	GCCTACCCCC CTATGGGGG	GGCTATCGTG CCGATAGCAC	GCTACACCTAL CGATGTGGAT	ASK 9999999
18051	AGACGAGCAA TCTGCTCGTT	CTACCCGACG GATGGGCTGC	CCGAACCACC GGCTTGGTGG	ACTGGAACCC TGACCTTGGG	cecceccec
18101	TCGCCGTCGC	CAGCCCGTGC	TGGCCCCGAT	TTCCGTGCGC	AGGGTGGCTC
	AGCGGCAGCG	GTCGGGCACG	ACCGGGGCTA	AAGGCACGCG	TCCCACCGAG
18151	GCGAAGGAGG	CAGGACCCTG	GTGCTGCCAA	CAGCGCGCTA	CCACCCCAGC
	CGCTTCCTCC	GTCCTGGGAC	CACGACGGTT	GTCGCGCGAT	GGTGGGGTCG
18201	ATCGTTTAAA	AGCCGGTCTT	TGTGGTTCTT	GCAGATATGG	CCCTCACCTG
	TAGCAAATTT	TCGGCCAGAA	ACACCAAGAA	CGTCTATACC	GGGAGTGGAC
18251	CCGCCTCCGT	TTCCCGGTGC	CGGGATTCCG	AGGAAGAATG	CACCGTAGGA
	GGCGGAGGCA	AAGGGCCACG	GCCCTAAGGC	TCCTTCTTAC	GTGGCATCCT
18301	CCCCATGCC CCCCATGCCC	CGGCCACGGC	CTGACGGGCG GACTGCCCGC	GCATGCGTCG CGTACGCAGC	TGCGCACCAC ACGCGTGGTG
18351	0000000000	GCGCGTCGCA CGCGCAGCGT	CCGTCGCATG GGCAGCGTAC	CGCGGCGGTA	TCCTGCCCCT AGGACGGGGA
18401	CCTTATTCCA GGAATAAGGT	CTGATCGCCG GACTAGCGGC	CGGCGATTGG GCCGCTAACC	CGCCGTGCCC	GGAATTGCAT CCTTAACGTA
18451	CCGTGGCCTT	GCAGGCGCAG	AGACACTGAT	TAAAAACAAG	TTGCATGTGG
	GGCACCGGAA	CGTCCGCGTC	TCTGTGACTA	ATTTTTGTTC	AACGTACACC
18501	AAAAATCAAA	ATAAAAAGTC	TGGACTCTCA	CGCTCGCTTG	GTCCTGTAAC
	TTTTTAGTTT	TATTTTTCAG	ACCTGAGAGT	GCGAGCGAAC	CAGGACATTG
18551	TATTITGTAG ATAAAACATC	AATGGAAGAC TTACCTTCTG	ATCAACTTTG TAGTTGAAAC	CGTCTCTGGC	CCCGCGACAC
18,601	GGCTCGCGCC CCGAGCGCGG	CCTTCATGGG GCAAGTACCC	AAACTGGCAA TTTGACCGTT	GATATCGGCA CTATAGCCGT	CCAGCAATAT GGTCGTTATA
18651	GAGCGGTGGC CTCGCCACCG	GCCTTCAGCT GCGGAAGTCGA	GGGGCTCGCT	CACCTCGCCG	TAAAAAATT TAATTTTAA
18701	TCGGTTCCAC	CGTTAAGAAC	TATGGCAGGA	AGGCCTGGAA	CAGCAGCACA
	AGCCAAGGTG	GCAATTCTTG	ATACCGTCGT	TCCGGACCTT	CTCGTCGTGT
18751	GGCCAGATGC CCGGTCTACC	TGAGGGATAA ACTCCCTATI	CAACTITCTC	CAAAATTTCC CTTTTAAAGG	: AACAAAAGGT : TTGTTTTCCA
18801	GGTAGATGGC	CTGGCCTCTG	GCATTAGCGG	GGTGGTGGAC	CTGGCCAACC
	CCATCTACCC	GACCGGAGAC	CGTAATCGCC	CCACCACCTG	GACCGGTTGG
18851	AGGCAGTGCA	TABAATAAGA <i>L</i>	AACAGTAAGO	TTGATCCCCG	CCCTCCCGTA
	TCCGTCACGT	LATOTTATTT 1	TTGTCATTCO	AACTAGGGGC	GGGAGGGCAT
18901	GAGGAGCCT(CACCGGCCGT	GGAGACAGTO	TCTCCAGAGG	GCCCTCCCA
	CTCCTCGGA(GTGGCCGGCI	CCTCTGTCAC	AGAGGTCTCC	CCCCACCCCT

Figure 26T

18951	AAAGCGTCCG TTTCGCAGGC	CCGACA GCGGGGCTGT	GGGAAGAAAC CCCTTCTTTG	TCTGGTGACG AGACCACTGC	CAAATA G
19001				AAGGCCTGCC TTCCGGACGG	
19051	CCCATCGCGC GGGTAGCGCG	-		GGCCAGCACA CCGGTCGTGT	
19101				GCAGAAACCT CGTCTTTGGA	
19151				GCCGCGCGTC CGGCGCGCAG	
19201				GTAGCCAGTG CATCGGTCAC	
19251				GGTGCAATCC CCACGTTAGG	
19301	GACGATGCTT CTGCTACGAA			TGTGTGTCAT ACACACAGTA	
19351			-	CCCCCCCCCC	
19401				CTTACATGCA GAATGTACGT	
19451				CTGGTGCAGT. GACCACGTCA	
19501				GTTTAGAAAC CAAATCTTTG	
19551				CCCAGCGTTT GGGTCGCAAA	
19601				TACTCGTACA ATGAGCATGT	
19651				GGACATGGCT CCTGTACCGA	
19701	TTGACATCCG AACTGTAGGC				GCCCTACTCT CGGGATGAGA
19751	GGCACTGCCT CCGTGACGGA			GGTGCCCCAA CCACGGGGTT	
19801	ATGGGATGAA TACCCTACTT			AAACCTAGAA TTTGGATCTT	
19851	ATGACAACGA TACTGTTGCT			CTGAGCAGCA GACTCGTCGT	

Figure 26 U

19901 .	GTATTTGGGC CATAAACCCG	A GCCTTA TCCGCGGAAT	TTCTGGTATA AAGACCATAT	AATATTACAA TTATAATGTT	AGGAGG T TCCTCCCATA
19951		GTCGAAGGTC CAGCTTCCAG			
20001		TCAAATAGGA AGTTTATCCT			
20051		GGAGAGTCCT CCTCTCAGGA			
20101		GCAAAACCCA CGTTTTGGGT			
20151		AAATGGAAAG TTTACCTTTC			
20201	TCAACTACTG AGTTGATGAC	AGGCAGCCGC	AGGCAATGGT TCCGTTACCA	GATAACTTGA CTATTGAACT	CTCCTAAAGT GAGGATTTCA
20251		AGTGAAGATG TCACTTCTAC			
20301		CACTATTAAG GTGATAATTC			
20351		CCAACAGGCC GGTTGTCCGG			
20401		TATTACAACA ATAATGTTGT			
20451		GTTGAATGCT CAACTTACGA			
20501		AGCTTTTGCT TCGAAAACGA			
20551					GTTAGAATTA CAATCTTAAT
20601					CTTTCCACTG GAAAGGTGAC
20651	GGAGGTGTGA CCTCCACACT	TTAATACAGA AATTATGTCT	GACTCTTACC CTGAGAATGG	AAGGTAAAAC TTCCATTTTG	CTAAAACAGG GATTTTGTCC
20701	TCAGGAAAAT AGTCCTTTTA	GGATGGGAAA CCTACCCTTT	AAGATGCTAC TTCTACGATG	AGAATTTTCA TCTTAAAAGT	GATAAAAATG CTATTTTTAC
20751					AAATGCCAAC TTTACGGTTG
20801	CTGTGGAGAA GACACCTCTT	ATTTCCTGTA TAAAGGACAT	CTCCAACATA GAGGTTGTAT	GCGCTGTATT CGCGACATAA	TGCCCGACAA ACGGGCTGTT

Figure 26 V

20851	GCTAAAGTAC CGATTTCATG	ACCTTCCA TEGAAGGT	ACGTAAAAAT TGCATTTTTA	TTCTGATÄÄČ AAGACTATTG	TCAAAC) T GCTTTG A
20901				CCGGGCTAGT GGCCCGATCA	
20951				TATATGGACA ATATACCTGT	
21001				CTACCGCTCA GATGGCGAGT	
21051				AGGTGCCTCA TCCACGGAGT	
21101				TCATACACCT AGTATGTGGA	
21151				GAGCTCCCTA CTCGAGGGAT	
21201				ATAGCATTTG TATCGTAAAC	
21251				TCCACGCTTG AGGTGCGAAC	
21301				CGACTATCTC GCTGATAGAG	
21351				CCAACGTGCC GGTTGCACGG	
21401	CCCTCCCGCA GGGAGGGCGT	ACTGGGCGGC TGACCCGCCG	TTTCCGCGGC AAAGGCGCCG	TGGGCCTTCA ACCCGGAAGT	CGCGCCTTAA GCGCGGAATT
21451				CTACGACCCT GATGCTGGGA	
21501				CCTTTTACCT GGAAAATGGA	
21551				TCTGTCAGCT AGACAGTCGA	GGCCTGGCAA CCGGACCGTT
21601					TCAGTTGACG AGTCAACTGC
21651	GGGAGGGTTA CCCTCCCAAT	CAACGTTGCC	CAGTGTAACA GTCACATTGT	TGACCAAAGA ACTGGTTTCT	CTGGTTCCTG GACCAAGGAC
21701	GTACAAATGC	TAGCTAACTA	TAACATTGGC	TACCAGGGCT	TCTATATCCC AGATATAGGG
21751	AGAGAGCTAC	AAGGACCGCA	TGTACTCCTT	CTTTAGAAAC	TTCCAGCCCA AAGGTCGGGT

Figure 26 W

21801	TGAGCCGTCA ACTCGGCAGT	CONCETT CACCTA	GATACŤAAAT CTATGATTTA	ACAAGGACTAL TGTTCCTGAT	CEPACACE C
21851				TTTGTTGGCT AAACAACCGA	
21901				TAACTTCCCC ATTGAAGGGG	
21951	TAGGCAAGAC ATCCGTTCTG	CGCAGTTGAC GCGTCAACTG	AGCATTACCC TCGTAATGGG	AGAAAAAGTT TCTTTTTCAA	TCTTTGCGAT AGAAACGCTA
22001				AACTTTATGT TTGAAATACA	
22051	ACTCACAGAC TGAGTGTCTG	CTGGGCCAAA GACCCGGTTT	ACCTTCTCTA TGGAAGAGAT	CGCCAACTCC GCGGTTGAGG	GCCCACGCGC CGGGTGCGCG
22101	TAGACATGAC ATCTGTACTG	TTTTGAGGTG AAAACTCCAC	GATCCCATGG CTAGGGTACC	ACGAGCCCAC TGCTCGGGTG	CCTTCTTTAT GGAAGAAATA
22151				GTGCACCAGC CACGTGGTCG	
22201				CTTCTCGGCC GAAGAGCCGG	
22251	CAACATAAAG GTTGTATTTC	AAGCAAGCAA TTCGTTCGTT	CATCAACAAC GTAGTTGTTG	AGCTGCCGCC TCGACGGCGG	ATGGGCTCCA TACCCGAGGT
22301	GTGAGCAGGA CACTCGTCCT	ACTGAAAGCC TGACTTTCGG	ATTGTCAAAG TAACAGTTTC	ATCTTGGTTG TAGAACCAAC	TGGGCCATAT ACCCGGTATA
22351				GGCTTTGTTT .CCGAAACAAA	
22401	GCTCGCCTGC CGAGCGGACG	GCCATAGTCA CGGTATCAGT	ATACGGCCGG TATGCCGGCC	TCGCGAGACT AGCGCTCTGA	GGGGGCGTAC
22451				CAAAAACATG GTTTTTGTAC	
22501				AAGCAGGTTT TTCGTCCAAA	
22551	GTACGAGTCA CATGCTCAGT	CTCCTGCGCC GAGGACGCGG	GTAGCGCCAT CATCGCGGTA	TGCTTCTTCC ACGAAGAAGG	CCCGACCGCT GGGCTGGCGA
22601	GTATAACGCT CATATTGCGA	GGAAAAGTCC CCTTTTCAGG	ACCCAAAGCG TGGGTTTCGC	TACAGGGGCC ATGTCCCCGG	CAACTCGGCC GTTGAGCCGG
22651	GCCTGTGGAC CGGACACCTG	TATTCTGCTG ATAAGACGAC	CATGTTTCTC GTACAAAGAG	CACGCCTTTG GTGCGGAAAC	CCAACTGGCC GGTTGACCGG
22701	CCAAACTCCC GGTTTGAGGG	ATGGATCACA TACCTAGTGT	ACCCCACCAT TGGGGTGGTA	GAACCTTATT CTTGGAATAA	ACCGGGGTAC TGGCCCCATG

Figure 26 X

22751	CCAACTCCAT GGTTGAGGTA	GCTCAACAGT CTTGTCA	CCCCAGGTAC GGGGTCCATG	AGCCCACGEAT TCGGGTGGGA	cecyecture edexocorrect
22801				CACTCGCCCT GTGAGCGGGA	
22851	CCACAGTGCG	CAGATTAGGA	GCGCCACTTC	TTTTTGTCAC	TTGAAAAACA
	GGTGTCACGC	GTCTAATCCT	CGCGGTGAAG	AAAAACAGTG	AACTTTTGT
22901				ATAAAGGCAA TATTTCCGTT	
22951	TTGTACACTC AACATGTGAG			ACCCTTGCCG TGGGAACGGC	
23001		AAGGGGTTCT TTCCCCAAGA	GCCGCGCATC CGGCGCGTAG	GCTATGCGCC CGATACGCGG	ACTGGCAGGG TGACCGTCCC
23051				ACTTAAACTC TGAATTTGAG	
23101				CACAGGCTGC GTGTCCGACG	
23151	000			CTTGAAGTCG GAACTTCAGC	
23201				CAGGGTTGCA GTCCCAACGT	
23251				AGCACGCTCT TCGTGCGAGA	
23301				CAGGGCGAAC GTCCCGCTTG	
23351				GCCCAGGCTT CGGGTCCGAA	
23401	TCGCACCGTA AGCGTGGCAT			TGCCCGGTCT ACGGGCCAGA	
23451	ATACAGCGCC TATGTCGCGG	TGCATAAAAG ACGTATTTTC	CCTTGATCTG GGAACTAGAC	CTTAAAAGCC GAATTTTCGG	ACCTGAGCCT TGGACTCGGA
23501	TTGCGCCTTC AACGCGGAAG				AAACTGATTG TTTGACTAAC
23551	GCCGGACAGG CGGCCTGTCC	CCGCGTCGTG GGCGCAGCAC	CACGCAGCAC GTGCGTCGTG	CTTGCGTCGG GAACGCAGCC	TGTTGGAGAT ACAACCTCTA
23601	CTGCACCACA GACGTGGTGT				GCCTTGCTAG CGGAACGATC
23651	ACTGCTCCTT TGACGAGGAA				ATCCATTTCA TAGGTAAAGT

Figure 26 Y

23701	ATCACGTGCT TAGTGCACGA	CATATTAT GGAATAAATA	CATAATGCTT GTATTACGAA	CCGTGTAGAC GGCACATCTG	ACTTAA TC
23751		TCAGCGCAGC AGTCGCGTCG			
23801	CGTGATGCTT GCACTACGAA	GTAGGTCACC CATCCAGTGG	TCTGCAAACG AGACGTTTGC	ACTGCAGGTA TGACGTCCAT	CGCCTGCAGG GCGGACGTCC
23851	AATCGCCCCA TTAGCGGGGT	TCATCGTCAC AGTAGCAGTG	AAAGGTCTTG TTTCCAGAAC	TTGCTGGTGA AACGACCACT	AGGTCAGCTG TCCAGTCGAC
23901	CAACCCGCGG	TGCTCCTCGT ACGAGGAGCA	TCAGCCAGGT AGTCGGTCCA	CTTGCATACG GAACGTATGC	GCCGCCAGAG CGGCGGTCTC
23951	CTTCCACTTG	GTCAGGCAGT CAGTCCGTCA	AGTTTGAAGT	TCGCCTTTAG	ATCGTTATCC
24001	ACGTGGTACT TGCACCATGA	TGTCCATCAG ACAGGTAGTC	CGCGCGCGCA GCGCGCGCGT	GCCTCCATGC CGGAGGTACG	CCTTCTCCCA GGAAGAGGGT
24051	CGCAGACACG GCGTCTGTGC	ATCGGCACAC TAGCCGTGTG	TCAGCGGGTT AGTCGCCCAA	CATCACCGTA GTAGTGGCAT	ATTTCACTTT TAAAGTGAAA
24101	CCGCTTCGCT GGCGAAGCGA	GGGCTCTTCC CCCGAGAAGG	TCTTCCTCTT AGAAGGAGAA	GCGTCCGCAT CGCAGGCGTA	ACCACGCGCC TGGTGCGCGG
24151	ACTGGGTCGT TGACCCAGCA	CTTCATTCAG GAAGTAAGTC	CCGCCGCACT	GTGCGCTTAC CACGCGAATG	CTCCTTTGCC GAGGAAACGG
24201	TACGAACTAA	TCGTGGCCAC	CCAACGACTT	TGGGTGGTAA	
24251	GTAGAAGAGA	AAGAAGGAGC	GACAGGTGCT	AATGGAGACC	
24301	GCGAGCCCGA	ACCCTCTTCC	CGCGAAGAAA	AAGAAGAACC	GCGCAATGGC CGCGTTACCG
24351	GTTTAGGCGG	CGGCTCCAGC	TACCGGCGCC	CGACCCACAC	CGCGGCACCA GCGCCGTGGT
24401	CGCGCAGAAC	ACTACTCAGA	AGGAGCAGGA	. GCCTGAGCTA	ACGCCGCCTC TGCGGCGGAG
	TAGGCGAAAA	AACCCCCGCG	GGCCCTCCG	CCGCCGCTGC	GGGACGGGGA CCCTGCCCCT
	GCTGTGCAGG	AGGTACCAAC	CCCCTGCAGC	: GCGGCGTGGC	CGTCCGCGCT CCAGGCGCGA
	GCCCCACCA	AAGCGCGACG	AGGAGAAGGG	CTGACCGGT	TTCCTTCTCC AAGGAAGAGG
24601	TATAGGCAGA ATATCCGTCT	AAAAGATCAT TTTTCTAGTA	GGAGTCAGTC CCTCAGTCAG	GAGAAGAAGG GCTCTTCTTCC	ACAGCCTAAC TGTCGGATTG

Figure 262

24651	CGCCCCTCT GCGGGGGAGA		CACCGATGCC CTGGCTACGG	
24701	CTACCACCTT GATGGTGGAA		TTGAGGAGGA AACTCCTCCT	
24751 ·			GACGACGAGG CTGCTGCTCC	
24801			CAACGCAGAG GTTGCGTCTC	
24851		 	GCGACTACCT CGCTGATGGA	
24901			CAGTGCGCCA GTCACGCGGT	
24951		 	CGCCATAGCG GCGGTATCGC	, .
25001			GCGTACCCCC CGCATGGGGG	
25051		 	CTCAACTTCT GAGTTGAAGA	
25101	TGCCGTGCCA ACGGCACGGT		CATCTTTTTC GTAGAAAAAG	
25151			GCCGAGCGGA CGGCTCGCCT	
25201		 	ATCGCCTCGC TAGCGGAGCG	
25251			CGAGAAGCGC GCTCTTCGCG	-
25301		 	GTCACTCTGG CAGTGAGACC	
25351		 	GTACTAAAAC CATGATTTTG	
25401	GGTCACCCAC CCAGTGGGTG		CCTACCCCC GGATGGGGGG	
25451	GCACAGTCAT CGTGTCAGTA		GTGCGCAGCC CACGCGTCGG	
25501	GATGCAAATT CTACGTTTAA		GGCCTACCCG CCGGATGGGC	
25551				GACTTGGAGG CTGAACCTCC

7 igure 26 AA

25601	AGCGACGCAA	AATGATG	GCCGCAGTGC	TCGTTACCGT	GGAGCT AG
	TCGCTGCGTT	TGATTACTAC	CGGCGTCACG	AGCAATGGCA	CCTCGAACTC
25651		GGTTCTTTGC CCAAGAAACG			
25701	AACATTGCAC	TACACCTTTC	GACAGGGCTA	CGTACGCCAG	GCCTGCAAGA
	TTGTAACGTG	ATGTGGAAAG	CTGTCCCGAT	GCATGCGGTC	CGGACGTTCT
25751	TCTCCAACGT	GGAGCTCTGC	AACCTGGTCT	CCTACCTTGG	AATTTTGCAC
	AGAGGTTGCA	CCTCGAGACG	TTGGACCAGA	GGATGGAACC	TTAAAACGTG
25801	GAAAACCGCC	TTGGGCAAAA	CGTGCTTCAT	TCCACGCTCA	AGGGCGAGGC
	CTTTTGGCGG	AACCCGTTTT	GCACGAAGTA	AGGTGCGAGT	TCCCGCTCCG
25851	CCCCCCCGAC	TACGTCCGCG ATGCAGGCGC	ACTGCGTTTA TGACGCAAAT	CTTATTTCTA GAATAAAGAT	TGCTACACCT ACGATGTGGA
25901	GGCAGACGGC	CATGGGCGTT	TGGCAGCAGT	GCTTGGAGGA	GTGCAACCTC
	CCGTCTGCCG	GTACCCGCAA	ACCGTCGTCA	CGAACCTCCT	CACGTTGGAG
25951		AGAAACTGCT TCTTTGACGA			
26001	CTTCAACGAG GAAGTTGCTC	CGCTCCGTGG GCGAGGCACC	CCGCGCACCT	GGCGGACATC CCGCCTGTAG	ATTTTCCCCG TAAAAGGGGC
26051	AACGCCTGCT	TAAAACCCTG	CAACAGGGTC	TGCCAGACTT	CACCAGTCAA
	TTGCGGACGA	ATTTTGGGAC	GTTGTCCCAG	ACGGTCTGAA	GTGGTCAGTT
26101	AGCATGTTGC	AGAACTTTAG	GAACTTTATC	CTAGAGCGCT	CAGGAATCTT
	TCGTACAACG	TCTTGAAATC	CTTGAAATAG	GATCTCGCGA	GTCCTTAGAA
26151	GCCCGCCACC	TGCTGTGCAC	TTCCTAGCGA	CTTTGTGCCC	ATTAAGTACC
	CGGGCGGTGG	ACGACACGTG	AAGGATCGCT	GAAACACGGG	TAATTCATGG
26201	GCGAATGCCC	TCCGCCGCTT	TGGGGCCACT	GCTACCTTCT	GCAGCTAGCC
	CGCTTACGGG	AGGCGGCGAA	ACCCCGGTGA	CGATGGAAGA	CGTCGATCGG
26251					GCGGTGACGG CGCCACTGCC
26301	TCTACTGGAG AGATGACCTC	TGTCACTGTC ACAGTGACAG	GCTGCAACCT CGACGTTGGA	TACGTGGGGG	CACCGCTCCC CTGGCGAGGG
26351	TGGTTTGCAA ACCAAACGTT	TTCGCAGCTG AAGCGTCGAC	CTTAACGAAA GAATTGCTTT	CAGTTTAATA	CGGTACCTTT CGCATGGAAA
26401	GAGCTGCAGG	GTCCCTCGCC	TGACGAAAAG	TCCGCGGCTC	CGGGGTTGAA
	CTCGACGTCC	CAGGGAGCGG	ACTGCTTTTC	AGGCGCCGAG	GCCCCAACTT
26451	ACTCACTCCC TGAGTGAGGC	GGGCTGTGGA CCCGACACCT	CGTCGGCTTA CCAGCCGAAT	CCTTCGCAAA GGAAGCGTTT	TTTGTACCTG AAACATGGAC
26501	AGGACTACCA	CGCCCACGAG	ATTAGGTTC1	ACGAAGACCA	A TCCCGCCCG
	TCCTGATGGT	CGCGGGTGCTC	TAATCCAAGA	TGCTTCTGGT	T TAGGGCGGGC

Figure 26 AB

26551		 	ACCCAGGGCC TGGGTCCCGG	
26601			AGAGTTTCTG TCTCAAAGAC	
26651		 	GCGAGGAGCT CGCTCCTCGA	
26701		 	Gececccee	
26751			CGCCGCCACC GCGGCGGTGG	
26801		 	GTTTTGGACG CAAAACCTGC	
26851			CGAGGAAGCT GCTCCTTCGA	
26901			CGGTCGCATT GCCAGCGTAA	
26951			ATGGCTACAA TACCGATGTT	
27001		 	ACCCAACCGT TGGGTTGGCA	
27051			AGCCGCCGCC TCGGCGGCGG	
27101		 	TGGCGCGGGC ACCGCGCCCG	
27151			CAACATCTCC GTTGTAGAGG	
27201		 - 1	TCCCCCGTAA AGGGGGCATT	
27251			ACCGGCGCCA TGGCCGCCGT	
27301	CAGCAGCGGC			
27351	AAGCCCAAGA TTCGGGTTCT		GCAGGAGGAG CGTCCTCCTC	
27401	TCTGGCGCCC AGACCGCGGG			AACAGGATTT TTGTCCTAAA
27451	TTCCCACTCT AAGGGTGAGA		GCAGGGGCCA CGTCCCCGGT	

Figure 26 AC

27501	CTGAAAATAA GACTTTTATT	A CAGGTC TITTGTCCAG	TCTGCGATCC AGACGCTAGG	CTCACCCGCA GAGTGGGCGT	GCTGCC 'A CGACGGACAT
27551		GAAGATCAGC CTTCTAGTCG			
27601		ATACTGCGCG [°] TATGACGCGC			
27651	TCTCAAATTT AGAGTTTAAA	AAGCGCGAAA TTCGCGCTTT			
27701		GTTGTCAGCG CAACAGTCGC			
27751	ACATGTGGAG TGTACACCTC	TTACCAGCCA AATGGTCGGT	CAAATGGGAC GTTTACCCTG	TTGCGGCTGG AACGCCGACC	AGCTGCCCAA TCGACGGGTT
27801		CCCGAATAAA GGGCTTATTT			
27851		GGAATACGCG CCTTATGCGC			
27901		CACCACACCT GTGGTGTGGA			
27951		TGTACCAGGA ACATGGTCCT			TGGTACTTCC ACCATGAAGG
28001		CAGGCCGAAG GTCCGGCTTC			GCGCAGCTTG CGCGTCGAAC
28051	CGGGCGGCTT	TCGTCACAGG AGCAGTGTCC	GTGCGGTCGC CACGCCAGCG	CCGGGCAGGG	TATAACTCAC ATATTGAGTG
28101	CTGACAATCA GACTGTTAGT	GAGGGCGAGG CTCCCGCTCC	TATTCAGCTC ATAAGTCGAG	AACGACGAGT TTGCTGCTCA	CGGTGAGCTC
28151					CCCCGCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
	CGAGAAGTAA	GTGCGGAGCA	GTCCGTTAGG	ATTGAGACGT	GACCTCGTCC CTGGAGCAGG
28251	TCTGAGCCGC	GCTCTGGAGG CGAGACCTCC	CATTGGAACT GTAACCTTGA	CTGCAATTTA GACGTTAAAT	TTGAGGAGTT AACTCCTCAA
28301	TGTGCCATCG ACACGGTAGC	GTCTACTTTA CAGATGAAAT	ACCCCTTCTC	GGGACCTCCC	GGCCACTATC CCGGTGATAG
28351	CGGATCAATT GCCTAGTTAA	TATTCCTAAC ATAAGGATTG	TTTGACGCGG	TAAAGGACTC	GGCGGACGGC CCGCCTGCCG
28401	TACGACTGAA ATGCTGACTT	TGTTAAGTGG ACAATTCACC	AGAGGCAGAG TCTCCGTCTC	CAACTGCGCC GTTGACGCGG	TGAAACACCT ACTTTGTGGA

Figure 26 AD

28451	GGTCCACTGT	CUCCGCCACA	AGTGCTTTGC	CCGCGACTCC	GGTGAG
				GGCGCTGAGG	
	condition.	0000003331	icucannuca	GGCGC1GAGG	court casas
28501		_		AGGGCCCGGC	
	CGATGAAACT	TAACGGGCTC	CTAGTATAGC	TCCCGGGCCG	CGTGCCGCAG
28551	CGGCTTACCG	CCCAGGGAGA	GCTTGCCCGT	AGCCTGATTC	GGGAGTTTAC
				TCGGACTAAG	
	GCCG-M-1 GGC	GGGTCCCTCT	COMMCOGGCM	ICOGACIAAG	CCCTCAAATG
28601				GGGACCCTGT	
	GGTCGCGGGG	GACGATCAAC	TCGCCCTGTC	CCCTGGGACA	CAAGAGTGAC
28651	TGATTTGCAA	CTGTCCTAAC	CCTGGATTAC	ATCAAGATCT	TTGTTGCCAT
••••				TAGTTCTAGA	
	NCINAMOGII	GUCUGGUIIG	GGACCIAAIG	INGIICIAGA	AACAACGGIA
28701	-			TAAAATATAC	
	GAGACACGAC	TCATATTATT	TATGTCTTTA	ATTITATATG	ACCCCGAGGA
			•		•
28751	ATCGCCATCC	TGTAAACGCC	ACCGTCTTCA	CCCGCCCAAG	CAAACCAAGG
	TAGCGGTAGG	ACATTTGCGG	TGGCAGAAGT	GGGCGGGTTC	GTTTGGTTCC
20001	OCA A OCEMBA C		ma a camemen	CCCTCTGTGA	mmm> C> > C> C
28801					
	GCTTGGAATG	GACCATGAAA	ATTGTAGAGA	GGGAGACACT	AAATGTTGTC
•					
28851	TTTCAACCCA	GACGGAGTGA	GTCTACGAGA	GAACCTCTCC	GAGCTCAGCT
	AAAGTTGGGT	CTGCCTCACT	CAGATGCTCT	CTTGGAGAGG	CTCGAGTCGA
28901	Δ CΤCCΔΤCΔC	אאאאארארר	אַרירי ייריריידיאַ	CCTGCCGGGA	ACGTACGAGT
20301				GGACGGCCCT	
	16AGG1AG1C	11111116166	TGGGAGGAAT	GGACGGCCCT	16CM16C1CM
28951				CCTGACCGTA	
	CGCAGTGGCC	GGCGACGTGG	TGTGGATGGC	GGACTGGCAT	TTGGTCTGAA
29001	TTTCCGGACA	GACCTCAATA	ACTCTGTTTA	CCAGAACAGG	AGGTGAGCTT
	AAAGGCCTGT	СТССАСТТАТ	TCACACAAAT	GGTCTTGTCC	ТССАСТССАА
			10/10/10/11		
20051	7C22220000	ma cocma mma	0000333000	002000200	macaamman m
29051				GCAGCTACTG	
	TCTTTTGGGA	ATCCCATAAT	CCGGTTTCCG	CGTCGATGAC	ACCCCAAATA
29101	GAACAATTCA	AGCAACTCTA	CGGGCTATTC	TAATTCAGGT	TTCTCTAGAA
	CTTGTTAAGT	TCGTTGAGAT	GCCCGATAAG	ATTAAGTCCA	AAGAGATCTT
			•		
29151	WCCCCCTTCC	ריבוואני א מאור _י מור	מבישרים שיים שיים שיים שיים שיים שיים שיים	TTCTCTTTAT	ጥር ተምስ ተመሰ
23131				AAGAGAAATA	
	AGCCCCAACC	CCAATAAGAG	ACAGAACACT	AAGAGAAATA	AGAATATGAT
		•			
29201	ACGCTTCTCT	GCCTAAGGCT	CGCCGCCTGC	TGTGTGCACA	TTTGCATTTA
	TGCGAAGAGA	CGGATTCCGA	GCGGCGGACG	ACACACGTGT	AAACGTAAAT
29251	JAIGHC VICTURE	TTTAAACGCT	GGGGTCGCCA	CCCAAGATGA	TTAGGTACAT
					AATCCATGTA
	PUCUO I CONN	www.iiocou	CCCCAGCGGI	COSTICIACI	anicunium
29301					ACCCAAAAGG
	TTAGGATCCA	AATGAGTGGG	AACGCAGTCG	GGTGCCATGG	TGGGTTTTCC
29351	TGGATTTTAA	GGAGCCAGCC	TGTAATGTTA	CATTCGCAGC	TGAAGCTAAT
	ACCTAAAATT	CCTCGGTCGG	ACATTACAAT	GTAAGCGTCG	ACTTCGATTA

Figure 26 AE

29401	GAGTGCACCA CTCACGTGGT	CTATAAA GAGAATATTT	ATGCACCACA TACGTGGTGT	GAACATGAAA CTTGTACTTT	AGCTGC T
29451	TCGCCACAAA AGCGGTGTTT	AACAAAATTG TTGTTTTAAC	GCAAGTATGC CGTTCATACG	TGTTTATGCT ACAAATACGA	ATTTGGCAGC TAAACCGTCG
29501	CAGGTGACAC GTCCACTGTG	TACAGAGTAT ATGTCTCATA	AATGTTACAG TTACAATGTC	TTTTCCAGGG AAAAGGTCCC	TAAAAGTCAT ATTTTCAGTA
29551	AAAACTTTTA TTTTGAAAAT	TGTATACTTT ACATATGAAA	TCCATTTAT AGGTAAAATA	GAAATGTGCG CTTTACACGC	ACATTACCAT TGTAATGGTA
29601	GTACATGAGC CATGTACTCG	AAACAGTATA TTTGTCATAT	AGTTGTGGCC TCAACACCGG	CCCACAAAAT GGGTGTTTTA	TGTGTGGAAA ACACACCTTT
29651		TTTCTGCTGC AAAGACGACG			GCTCGCTTTG CGAGCGAAAC
29701	CAGACATGGG	TACTCTATAT ATGAGATATA	ATTTATGTTT	TCGTCTGCGT	CGAAATAACT
29751	GGAAAAGAAA CCTTTTCTTT	ATGCCTTAAT TACGGAATTA	TTACTAAGTT AATGATTCAA	ACAAAGCTAA TGTTTCGATT	TGTCACCACT ACAGTGGTGA
29801	TTGACGAAAT	GAGCGACGAA	CGTTTTGTTT	AAGTTTTTCA	
29851		CTAAATTTGG	GGGGCCAGTA	AAGGACGAGT	TATGGTAAGG
29901	GGACTTGTTA	ACTGAGATAC	ACCCTATACG	AGGTCGCGAT	CAACCTTGAA GTTGGAACTT
29951	CAGTCCGAAG	CTGGATGTCA GACCTACAGT	CGTAGACTGA	AACCGGTCGT	GGACAGGGCG
30001	CCTAAACAAG	GTCAGGTTGA	TCTCGCTGGG	TGGGATTGTC	AGATGACCAA TCTACTGGTT
30051	CACAACCAAC GTGTTGGTTG	CCCCCCCCC	CTACCGGACT GATGGCCTGA	TACATCTACC ATGTAGATGG	ACAAATACAC TGTTTATGTG
30101	CCCAAGTTTC GGGTTCAAAG	TGCCTTTGTC	AATAACTGGG TTATTGACCC	ATAACTTGGG TATTGAACCC	CATGTGGTGG GTACACCACC
30151	TTCTCCATAG AAGAGGTATC	CGCTTATGTT CCGAATACAA	TGTATGCCTT	ATTATTATGT TAATAATACA	GGCTCATCTG CCGAGTAGAC
30201	CTGCCTAAAG GACGGATTTC	CGCAAACGCG	CCCGACCACC	CATCTATAGT GTAGATATCA	CCCATCATTG GGGTAGTAAC
30251	TGCTACACCO ACGATGTGGG	AAACAATGAT TTTGTTACTA	GGAATCCATA CCTTAGGTAT	CTAACCTGCC	ACTGAAACAC TGACTTTGTG
30301	ATGTTCTTTT TACAAGAAAA	CTCTTACAGT GAGAATGTCA	ATGATTAAAT TACTAATTTA	GAGACATGAT CTCTGTACTA	TCCTCGAGTT AGGAGCTCAA

Figure 26 AF

30351					CCACATTEC.
	AAATATAATG	ACTGGGAACA	ACGCGAAAAA	ACACGCACGA	GGTGTAACCG
30401				TCCAGCCTTC AGGTCGGAAG	
30451				TCTGCAGCCT AGACGTCGGA	
	2002211000				
30501				GTCTGTGTGC	
	CAGTAGCGGA	MAIAGGICAC	GIMACIGACC	CAGACACACG	CGAAACGTAT
30551	TCTCAGACAC	CATCCCCAGT	ACAGGGACAG	GACTATAGCT	GAGCTTCTTA
	AGAGTCTGTG	GTAGGGGTCA	TGTCCCTGTC	CTGATATCGA	CTCGAAGAAT
30601				TTTTCTGCTG	
	CTTAAGAAAT	TAATACTTTA	AATGACACTG	AAAAGACGAC	TAATAAACGT
30651				AGCCTCAAAG	
	GGGATAGACG	CAAAACAAGG	GGCTGGAGGT	TCGGAGTTTC	TGTATATAGT
30701				AGTTGCTACA	
	ACGTCTAAGT	GAGCATATAC	CTTATAAGGT	TCAACGATGT	TACTTTTTTC
30751	CGATCTTTCC	GAAGCCTGGT	TATATGCAAT	CATCTCTGTT	ATGGTGTTCT
	GCTAGAAAGG	CTTCGGACCA	ATATACGTTA	GTAGAGACAA	TACCACAAGA
30801	GCAGTACCAT	CTTAGCCCTA	GCTATATATC	CCTACCTTGA	CATTGGCTGG
	CGTCATGGTA	GAATCGGGAT	CGATATATAG	GGATGGAACT	GTAACCGACC
30851	AACGCAATAG	ATGCCATGAA	CCACCCAACT	TTCCCCGCGC	CCGCTATGCT
	TTGCGTTATC	TACGGTACTT	GGTGGGTTGA	AAGGGGCGCG	GGCGATACGA
30901				TGTCCCAGCC	
	AGGTGACGTT	GTTCAACAAC	GGCCGCCGAA	ACAGGGTCGG	TTAGTCGGAG
30951				GCTACTTTAA	
	CGGGTGGAAG	AGGGTGGGGG	TGACTTTAGT	CGATGAAATT	AGATTGTCCT
31001				GGACGGAATT	
	CCTCTACTGA	CTGTGGGÀTC	TAGATCTTTA	CCTGCCTTAA	TAATGTCTCG
31051				CCGAGCAACA	
	TCGCGGACGA	TCTTTCTGCG	TCCCGTCGCC	GGCTCGTTGT	CGCGTACTTA
31101					GGGGTATCTT
	GTTCTCGAGG	TTCTGTACCA	ATTGAACGTG	GTCACGTTTT	CCCCATAGAA
31151					ACCACCGGAC
	AACAGAGCAT	TTCGTCCGGT	TTCAGTGGAT	GCTGTCATTA	TGGTGGCCTG
31201					GCTGGTCATG
	TGGCGGAATC	GATGTTCAAC	GCTTGGTTCG	CAGTCTTTAA	CCACCAGTAC
31251	GTGGGAGAAA	AGCCCATTAC	CATAACTCAG	CACTCGGTAG	AAACCGAAGG
	CACCCTCTTT	TCGGGTAATG	GTATTGAGTC	GTGAGCCATC	TTTGGCTTCC

Figure 26 A6

31301	CTGCATTCAC	TCTTGTC	AAGGACCTGA	GGATCTCTGC	ACCCTT TA
	GACGTAAGTG	AGTGGAACAG	TTCCTGGACT	CCTAGAGACG	TGGGAATAAT
31351	AGACCCTGTG	CGGTCTCAAA	GATCTTATTC	CCTTTAACTA	AAAAAAAA
	TCTGGGACAC	GCCAGAGTTT	CTAGAATAAG	GGAAATTGAT	TTTTTTTAT
31401	ATAATAAAGC	ATCACTTACT	TAAAATCAGT	TAGCAAATTT	CTGTCCAGTT
	TATTATTTCG	TAGTGAATGA	ATTTTAGTCA	ATCGTTTAAA	GACAGGTCAA
31451		CACCTCCTTG GTGGAGGAAC			
31501	CTCCTGGCTG	CAAACTTTCT	CCACAATCTA	AATGGAATGT	CAGTTTCCTC
	GAGGACCGAC	GTTTGAAAGA	GGTGTTAGAT	TTACCTTACA	GTCAAAGGAG
31551	CTGTTCCTGT	CCATCCGCAC	CCACTATCTT	CATGTTGTTG	CAGATGAAGC
	GACAAGGACA	GGTAGGCGTG	GGTGATAGAA	GTACAACAAC	GTCTACTTCG
31601	GCGCAAGACC	GTCTGAAGAT	ACCTTCAACC	CCGTGTATCC	ATATGACACG
	CGCGTTCTGG	CAGACTTCTA	TGGAAGTTGG	GGCACATAGG	TATACTGTGC
31651	GAAACCGGTC	CTCCAACTGT	GCCTTTTCTT	ACTCCTCCCT	TTGTATCCCC
	CTTTGGCCAG	GAGGTTGACA	CGGAAAAGAA	TGAGGAGGGA	AACATAGGGG
31701		CAAGAGAGTC GTTCTCTCAG			
31751	AACCTCTAGT	TACCTCCAAT	GGCATGCTTG	CGCTCAAAAT	GGGCAACGGC
	TTGGAGATCA	ATGGAGGTTA	CCGTACGAAC	GCGAGTTTTA	CCCGTTGCCG
31801	CTCTCTCTGG GAGAGAGACC	ACGAGGCCGG TGCTCCGGCC	CAACCTTACC	TCCCAAAATG AGGGTTTTAC	TAACCACTGT ATTGGTGACA
31851	GAGCCCACCT	CTCAAAAAA	CCAAGTCAAA	CATAAACCTG	GAAATATCTG
	CTCGGGTGGA	GAGTTTTTTT	GGTTCAGTTT	GTATTTGGAC	CTTTATAGAC
31901	CACCCCTCAC GTGGGGAGTG	AGTTACCTCA TCAATGGAGT	GAAGCCCTAA	CTGTGGCTGC	CGCCGCACCT
31951	CTAATGGTCG GATTACCAGC	CGGGCAACAC	ACTCACCATG TGAGTGGTAC	CAATCACAGG GTTAGTGTCC	CCCCGCTAAC
32001	CGTGCACGAC GCACGTGCTG	TCCAAACTTA AGGTTTGAAT	GCATTGCCAC	CCAAGGACCC	CTCACAGTGT GAGTGTCACA
32051	CAGAAGGAAA	GCTAGCCCTG	CAAACATCAG	GCCCCTCAC	CACCACCGAT
	GTCTTCCTTT	CGATCGGGAC	GTTTGTAGTC	CGGGGGAGTG	GTGGTGGCTA
32101	AGCAGTACCO TCGTCATGGG	TTACTATCAC AATGATAGTG	TGCCTCACCC	GGAGATTGAT	CTGCCACTGG GACGGTGACC
32151	TAGCTTGGGC	ATTGACTTGA	AAGAGCCCAT	TTATACACAA	AATGGAAAAC
	ATCGAACCC	TAACTGAACT	TTCTCGGGTA	AATATGTGTT	TTACCTTTTG
32201	TAGGACTAA?	GTACGGGGCT	CCTTTCCATC	TAACAGACGA	CCTAAACACT
	ATCCTGATT	CATGCCCCGA	GGAAACGTAC	ATTGTCTGCT	GGATTTGTGA

Figure 26 AH

32251	TTGACCGTAG AACTGGCATC	CTGGTCC	AGGTGTGACT TCCACACTGA	ATTAATAATA TAATTAAT	CTTCCTTCA GAAGGA GT
32301				TTCACAAGGC AAGTGTTCCG	
32351	TTAATGTAGC AATTACATCG				
32401				AACCAACTAA TTGGTTGATT	
32451	•			CCACAACTTG GGTGTTGAAC	
32501				CAAACAATTC GTTTGTTAAG	
32551				ATGTTTGACG TACAAACTGC	
32601				TGGTTCACCT ACCAAGTGGA	
32651				ATGGCCTAGA TACCGGATCT	
32701				GGCCTTAGTT CCGGAATCAA	
3,2751				TGATAAGCTA ACTATTCGAT	
32801				TAAATGCAGA ATTTACGTCT	
32851				AGTCAAATAC TCAGTTTATG	
32901				TCCAATATCT AGGTTATAGA	
32951				AAAATGGAGT TTTTACCTCA	
33001	AATTCCTTCC TTAAGGAAGG				GAGATCTTAC CTCTAGAATG
33051	TGAAGGCACA ACTTCCGTGT	GCCTATACAA CGGATATGTT	ACGCTGTTGG TGCGACAACC	ATTTATGCCT TAAATACGGA	AACCTATCAG TTGGATAGTC
33101	CTTATCCAAA GAATAGGTTT				TGTCAGTCAA ACAGTCAGTT
33151					CCATTACACT GGTAATGTGA

Figure 26 AI

33201	NN NCCCTACA	CENNACAG	CACACACAAC	TCCAAGTGCA	TACTO
33201	TTTGCCATGT	GRECTTTGTC	CTCTGTGTTG	AGGTTCACGT	ATGAGA CA
33251	CATTTTCATG	GGACTGGTCT	GGCCACAACT	ACATTAATGA	AATATTTGCC
	GTAAAAGTAC	CCTGACCAGA	CCGGTGTTGA	TGTAATTACT	TTATAAACGG
33301				CAAGAATAAA	
	TGTAGGAGAA	TGTGAAAAAG	TATGTAACGG	GTTCTTATTT	CTTAGCAAAC
33351					
•	ACAATACAAA	GTTGCACAAA	TAAAAAGTTA	ACGTCTTTTA	AAGTTCAGTA
33401	TTTTCATTCA	GTAGTATAGC	CCCACCACCA	CATAGCTTAT	ACAGATCACC
	AAAAGTAAGT	CATCATATCG	CCCTCCTCCT	GTATCGAATA	TGTCTAGTGG
33451	GTACCTTAAT	CAAACTCACA	GAACCCTAGT	ATTCAACCTG	CCACCTCCCT
	CATGGAATTA	GTTTGAGTGT	CTTGGGATCA	TAAGTTGGAC	GGTGGAGGGA
33501				CCCGGCTGGC	
	GGGTTGTGTG	TCTCATGTGT	CAGGAAAGAG	GGGCCGACCG	GAATTTTTCG
33551				GGTGTTATAT	
	TAGTATAGTA	CCCATTGTCT	GTATAAGAAT	CCACAATATA	AGGTGTGCCA
33601				ATTAATAAAC	•
	AAGGACAGCT	CGGTTTGCGA	GTAGTCACTA	TAATTATTTG	AGGGGCCCGT
33651				GCTGAGCCAC	
				CGACTCGGTG	
33701				GGAGAAGTCC	
				CCTCTTCAGG	*
33751				AGGGCGGTGG	
				TCCCGCCACC	
33801	••••			CCGTCCTGCA	
				GGCAGGACGT	
33851				ACCGCCCGCA	
				TGGCGGGCGT	
33901	CCTTGTCCTC	CGGGCACAGC	AGCGCACCCT	GATCTCACTT	AAATCAGCAC
				CTAGAGTGAA	
33951					ACAGTGCAAG
					TGTCACGTTC
34001	GCGCTGTATC	CAAAGCTCAT	GGCGGGGACC	ACAGAACCCA	CGTGGCCATC
					GCACCGGTAG
34051	ATACCACAAG	CGCAGGTAGA	TTAAGTGGCG	ACCCCTCATA	AACACGCTGG
					TTGTGCGACC
34101	ACATAAACAT	TACCTCTTTT	GGCATGTTGT	AATTCACCAC	CTCCCGGTAC
	TGTATTTGTA	ATGGAGAAAA	CCGTACAACA	TTAAGTGGTG	GAGGGCCATG

Figure 26 AJ

34151	CATATAAACC GTATATTTGG	T CATTAAA A CTAATTT	CATGGCGCCA GTACCGCGGT	TCCACCACCAT AGGTGGTGGT	TCCTAA CA
34201				CTGCAGGGAA	
34251				GACGTCCCTT	
34232				TTGGTACCTA	
34301				CACACGTGCA GTGTGCACGT	
34351				CATATCCCAG GTATAGGGTC	
34401				AGGGAAGACC TCCCTTCTGG	
34451				TCGGGCAGCA AGCCCGTCGT	
34501				AAAAGGAGGT TTTTCCTCCA	
34551				ATCGTGTTGG TAGCACAACC	
34601				TTTCCTGAAG AAAGGACTTC	
34651				GGTCTCGCCG CCAGAGCGGC	
34701				CTCAAAGCAT GAGTTTCGTA	CCAGGCGCCC GGTCCGCGGG
34751					GCCCTGATAA CGGGACTATT
34801					ACATTCGTTC TGTAAGCAAG
34851					CCATGTTTTT GGTACAAAAA
34901					ATCTATTAAG TAGATAATTC
34951					CCAAAGAACA GGTTTCTTGT
35001					AGGCAAACGG TCCGTTTGCC
35051					GTGAATCTCC CACTTAGAGG

Figure 26AK

35101	TCTATAAACA AGATATTTGT	TAGCACC AAGGTCGTGG	TTCAACCATG AAGTTGGTAC	CCCAAATAAT GGGTTTATTA	TCTCAT G AGAGTAGAGC
35151				CCGAATATTA GGCTTATAAT	
35201		•••		CCTTCAGCCT GGAAGTCGGA	
35251				AGACCTGTAT TCTGGACATA	
35301				CGTAGGTCCC GCATCCAGGG	
35351				GACCAGCGCG CTGGTCGCGC	
35401	CGCCAGGAAC GCGGTCCTTG	Catgacaaaa Gtactgtttt	GAACCCACAC CTTGGGTGTG	TGATTATGAC ACTAATACTG	ACGCATACTC TGCGTATGAG
35451				TAAGCTTGTT ATTCGAACAA	
35501				ATCAGGCAAA TAGTCCGTTT	
35551				GCAGATAAAG CGTCTATTTC	
35601				TTTCTCTCAA AAAGAGAGTT	
35651				CAAAAAAACA GTTTTTTTGT	TTTAAACATT AAATTTGTAA
35701	AGAAGCCTGT TCTTCGGACA	CTTACAACAG GAATGTTGTC	GAAAAACAAC CTTTTTGTTG	CCTTATAAGC GGAATATTCG	ATAAGACGGA TATTCTGCCT
35751					GTGATTAAAA CACTAATTTT
35801					TGTAAGACTC ACATTCTGAG
35851	GGTAAACACA CCATTTGTGT	TCAGGTTGAT AGTCCAACTA	TCACATCGGT AGTGTAGCCA	CAGTGCTAAA GTCACGATTT	AAGCGACCGA TTCGCTGGCT
35901	AATAGCCCGG TTATCGGGCC	GGGAATACAT CCCTTATGTA	ACCCGCAGGC TGGGCGTCCG	GTAGAGACAA CATCTCTGTT	CATTACAGCC GTAATGTCGG
35951	CCCATAGGAG GGGTATCCTC	GTATAACAAA CATATTGTTT	ATTAATAGGA TAATTATCCT	GAGAAAAACA CTCTTTTTGT	CATAAACACC GTATTTGTGG
36001	TGAAAAACCC ACTTTTTGGG	TCCTGCCTAG AGGACGGATC	GCAAAATAGC CGTTTTATCG	ACCCTCCCGC TGGGAGGGCG	TCCAGAACAA AGGTCTTGTT

Figure 26 AL

36051	CATACAGCGC GTATGTCGCG			CAGTCAGCCT GTCAGTCGGA	
36101				ACACGGCACC TGTGCCGTGG	
36151				GCGAGTATAT CGCTCATATA	
36201				AACACCCAGA TTGTGGGTCT	
36251	CGAACCTACG GCTTGGATGC			AACCCACAAC TTGGGTGTTG	
36301	CGTCACTTCC GCAGTGAAGG	GTTTTCCCAC CAAAAGGGTG	GTTACGTCAC CAATGCAGTG	TTCCCATTTT AAGGGTAAAA	AAGAAAACTA TTCTTTTGAT
36351				CTAAAACCTA GATTTTGGAT	
36401				ACTCCACCCC TGAGGTGGGG	
•					PacI
36451				ATTGATGATG TAACTACTAC	
36501	ATTCGGATCT TAAGCCTAGA	GCGACGCGAG CGCTGCGCTC	GCTGGATGGC CGACCTACCG	CTTCCCCATT GAAGGGGTAA	ATGATTCTTC TACTAAGAAG
36551	TCGCTTCCGG AGCGAAGGCC	CGGCATCGGG GCCGTAGCCC	ATGCCCGCGT TACGGGCGCA	TGCAGGCCAȚ ACGTCCGGTA	GCTGTCCAGG CGACAGGTCC
36601				CAAGGCCAGC GTTCCGGTCG	AAAAGGCCAG TTTTCCGGTC
36651					CTCCGCCCCC GAGGCGGGGG
36701					GCGAAACCCG CGCTTTGGGC
36751					CCCTCGTGCG
36801	CTCTCTGTT GAGAGACAA	CCGACCCTGC GGCTGGGACG	CGCTTACCGG GCGAATGGCC	ATACCTGTCC TATGGACAGG	GCCTTTCTCC CGGAAAGAGG
36851	CTTCGGGAAG GAAGCCCTTC	CGTGGCGCTT GCACCGCGAA	TCTCATAGCT AGAGTATCGA	CACGCTGTAG	GTATCTCAGT CATAGAGTCA
36901	TCGGTGTAGG AGCCACATCC	TCGTTCGCTC AGCAAGCGAG	CAAGCTGGGC GTTCGACCCG	TGTGTGCACG ACACACGTGC	AACCCCCCGT TTGGGGGGCA

Figure 26 AM

2.051	marcococae,		TATCCGGTAA	ርተልፕሮርፕሮፕፕ	GAGTCG
36951	AGTCGGGCTG	GEGACGCGGA	ATAGGCCATT	CATAGCAGAA	CTCAGGGG
37001	CGGTAAGACA GCCATTCTGT	CGACTTATCG GCTGAATAGC	CCACTGGCAG GGTGACCGTC	CAGCCACTGG GTCGGTGACC	TAACAGGATT ATTGTCCTAA
37051	AGCAGAGCGA	GGTATGTAGG	CGGTGCTACA GCCACGATGT	GAGTTCTTGA CTCAAGAACT	AGTGGTGGCC TCACCACCGG
37101	TAACTACGGC	TACACTAGAA	GGACAGTATT	TGGTATCTGC	GCTCTGCTGA
			CCTGTCATAA		
37151	AGCCAGTTAC TCGGTCAATG	CTTCGGAAAA GAAGCCTTTT	AGAGTTGGTA TCTCAACCAT	GCTCTTGATC CGAGAACTAG	CGGCAAACAA GCCGTTTGTT
37201	ACCACCGCTG TGGTGGCGAC	GTAGCGGTGG CATCGCCACC	TTTTTTTGTT AAAAAAACAA	TGCAAGCAGC ACGTTCGTCG	AGATTACGCG TCTAATGCGC
37251			AAGATCCTTT TTCTAGGAAA		
37301	ACGCTCAGTG	GAACGAAAAC	TCACGTTAAG	GGATTTTGGT	CATGAGATTA
			AGTGCAATTC		
37351	'TCAAAAAGGA AGTTTTTCCT	AGAAGTGGAT	GATCCTTTTA CTAGGAAAAT	TTAGTTAGAT	TTCATATATA
37401	GAGTAAACTT CTCATTTGAA	GGTCTGACAG CCAGACTGTC	TTACCAATGC AATGGTTACG	TTAATCAGTG AATTAGTCAC	AGGCACCTAT TCCGTGGATA
37451			GTTCATCCAT CAAGTAGGTA		
37501	TGTAGATAAC ACATCTATTG	TACGATACGG ATGCTATGCC	GAGGGCTTAC CTCCCGAATG	CATCTGGCCC GTAGACCGGG	CAGTGCTGCA GTCACGACGT
37551	ATGATACCGC TACTATGGCG	GAGACCCACG CTCTGGGTGC	CTCACCGGCT	CCAGATTTAT GGTCTAAATA	CAGCAATAAA GTCGTTATTT
37601	CCAGCCAGCC	GGAAGGGCCG	AGCGCAGAAG	TGGTCCTGCA ACCAGGACGT	ACTTTATCCG TGAAATAGGC
37651	CCTCCATCCA	GTCTATTAAT	TGTTGCCGGG	AAGCTAGAGT	AAGTAGTTCG
					TTCATCAAGC GCATCGTGGT
	GGTCAATTAT	CAAACGCGTT	GCAACAACGG	TAACGATGTC	CGTAGCACCA
37751	GTCACGCTCG CAGTGCGAGC	TCGTTTGGTA AGCAAACCAT	TGGCTTCATT ACCGAAGTAA	CAGCTCCGGT GTCGAGGCCA	TCCCAACGAT AGGGTTGCTA
37801	CAAGGCGAGT GTTCCGCTCA	TACATGATCO ATGTACTAGG	CCCATGTTGT GGGTACAACA	GCAAAAAAGC CGTTTTTCG	GGTTAGCTCC CCAATCGAGG
37851	TTCGGTCCTC AAGCCAGGAG	CGATCGTTGT	CAGAAGTAAG A GTCTTCATTC	TTGGCCGCAG	TGTTATCACT ACAATAGTGA

Figure 26 AN

37901	CATGGTTATG GTACCAATAC	CETCGTGACG	ATAATTCTCT TATTAAGAGA	TACTGTCATG ATGACAGTAC	CCATCO A CGTAGGLATT
37951	GATGCTTTTC	TGTGACTGGT	GAGTACTCAA	CCAAGTCATT	CTGAGAATAG
5,702				GGTTCAGTAA	
			•		
38001	TGTATGCGGC	GACCGAGTTG	CTCTTGCCCG	GCGTCAACAC	GGGATAATAC
	ACATACGCCG	CTGGCTCAAC	GAGAACGGGC	CGCAGTTGTG	CCCTATTATG
38051	CGCGCCACAT	AGCAGAACTT	TAAAAGTGCT	CATCATTGGA	AAACGTTCTT
	GCGCGGTGTA	TCGTCTTGAA	ATTTTCACGA	GTAGTAACCT	TTTGCAAGAA
38101				TGTTGAGATC	•
	GCCCCGCTTT	TGAGAGTTCC	TAGAATGGCG	ACAACTCTAG	GTCAAGCTAC
20151	mx > 000 > 000	CDCC2 CCC2 2	ODG NOODG N	GCATCTTTTA	COMPOS COSC
38151				CGTAGAAAAT	
	ATTGGGTGAG	CACGIGGGII	GACTAGAAGT	CGIMGMAMAI	GAMAGIGGIC
38201	CCTTTCTCCC	TCACCAAAAA	CAGGAAGGCA	AAATGCCGCA	AAAAAGGGAA
30201				TTTACGGCGT	
			0.00		
38251	TAAGGGCGAC	ACGGAAATGT	TGAATACTCA	TACTCTTCCT	TTTTCAATAT
				ATGAGAAGGA	
38301				ATGAGCGGAT	
	ATAACTTCGT	AAATAGTCCC	AATAACAGAG	TACTCGCCTA	TGTATAAACT
	v				
38351	ATGTATTTAG				
	TACATAAATC	TTTTTATTTG	TTTATCCCCA	AGGCGCGTGT	AAAGGGGCTT
					. mm
38401					ATTAACCTAT
	TTCACGGTGG	ACTGCAGATT	CTTTGGTAAT	AATAGTACTG	TAATTGGATA
38451	33333000CC	CWDWCDCCDC	CCCCTTTCCT	ריייר א א כא איי	TGGATCCGAA
20421					ACCTAGGCTT
	11111AICCG	CHINGIGCIC	CGGGRANGCA	GHOITCIIN	
	-	PacI			
38501	TTCTTAATTT	רתית בתית ב	(SEO TO NO	.32)	
20201	c.inniii		1000 00 110		

AAGAATTAAA GAATTAATT (SEQ ID NO:33)

Figure 26 AO

MRKAd5nef MER1063 (MRKAd5 Pre-Adenoviral Vector Containing the G2A,LLA nef Coding Region)

1	CATCATCAAT	AATATACCTT	ATTTTGGATT	GAAGCCAATA	TGATAATGAG
	•	TTATATGGAA			
51	GGGGTGGAGT	TTGTGACGTG	GCGCGGGGGG	TGGGAACGGG	GCGGGTGACG
	CCCCACCTCA	AACACTGCAC	CGCGCCCCGC	ACCCTTGCCC	CGCCCACTGC
101	TAGTAGTGTG	GCGGAAGTGT	GATGTTGCAA	GTGTGGCGGA	ACACATGTAA
	ATCATCACAC	CGCCTTCACA	CTACAACGTT	CACACCGCCT	TGTGTACATT
151	GCGACGGATG	TGGCAAAAGT	GACGTTTTTG	GTGTGCGCCG	GTGTACACAG
	CGCTGCCTAC	ACCGTTTTCA	CTGCAAAAAC	CACACGCGGC	CACATGTGTC
201	GAAGTGACAA	TTTTCGCGCG	GTTTTAGGCG	GATGTTGTAG	TAAATTTGGG
	CTTCACTGTT	AAAAGCGCGC	CAAAATCCGC	CTACAACATC	ATTTAAACCC
251	CGTAACCGAG	TAAGATTTGG	CCATTTTCGC	GGGAAAACTG	AATAAGAGGA
	GCATTGGCTC	ATTCTAAACC	GGTAAAAGCG	CCCTTTTGAC	TTATTCTCCT
301	AGTGAAATCT	GAATAATTTT	GTGTTACTCA	TAGCGCGTAA	TATTTGTCTA
	TCACTTTAGA	CTTATTAAAA	CACAATGAGT	ATCGCGCATT	ATAAACAGAT
351	GGGCCGCGG	GACTTTGACC	GTTTACGTGG	AGACTCGCCC	AGGTGTTTTT
	CCCGGCGCCC	CTGAAACTGG	CAAATGCACC	TCTGAGCGGG	TCCACAAAAA
401	CTCAGGTGTT	TTCCGCGTTC	CGGGTCAAAG	TTGGCGTTTT	ATTATTATAG
	GAGTCCACAA	AAGGCGCAAG	GCCCAGTTTC	AACCGCAAAA	TAATAATATC
451	GCGGCCGCGA	TCCATTGCAT	ACGTTGTATC	CATATCATAA	TATGTACATT
	CGCCGGCGCT	AGGTAACGTA	TGCAACATAG	GTATAGTATT	ATACATGTAA
501	TATATTGGCT	CATGTCCAAC	ATTACCGCCA	TGTTGACATT	GATTATTGAC
		GTACAGGTTG			
551	TAGTTATTAA	TAGTAATCAA	TTACGGGGTC	ATTAGTTCAT	AGCCCATATA
		ATCATTAGTT			
601	TGGAGTTCCG	CGTTACATAA	CTTACGGTAA	ATGGCCCGCC	TGGCTGACCG
	ACCTCAAGGC	GCAATGTATT	GAATGCCATT	TACCGGGCGG	ACCGACTGGC
651	CCCAACGACC	CCCGCCCATT	GACGTCAATA	ATGACGTATG	TTCCCATAGT
	GGGTTGCTGG	GGGCGGGTAA	CTGCAGTTAT	TACTGCATAC	AAGGGTATCA
701	AACGCCAATA	GGGACTTTCC	ATTGACGTCA	ATGGGTGGAG	TATTTACGGT
	TTGCGGTTAT	CCCTGAAAGG	TAACTGCAGT	TACCCACCTC	ATAAATGCCA
751	AAACTGCCCA	CTTGGCAGTA	CATCAAGTGT	ATCATATGCC	AAGTACGCCC
		GAACCGTCAT			
801	CCTATTGACG	TCAATGACGG	TAAATGGCCC	GCCTGGCATT	ATGCCCAGTA
	CCATAACTCC	AGTTACTGCC	ATTTACCGGG	CGGACCGTAA	TACGGGTCAT

Figure 27A

851				GTACATCTAC CATGTAGATG	
901	,			AGTACATCAA	
•	AGCGATAATG	GTACCACTAC	GCCAAAACCG	TCATGTAGTT	ACCCGCACCT
951				CTCCACCCCA GAGGTGGGGT	
1001				GGACTTTCCA CCTGAAAGGT	
1051				GTAGGCGTGT CATCCGCACA	
1101				CGTCAGATCG GCAGTCTAGC	
1151				ACACCGGGAC TGTGGCCCTG	
1201				GGATTCCCCG CCTAAGGGGC	
1251				CAAGAGGTCC GTTCTCCAGG	
1301				CCGAGCCCGC GGCTCGGGCG	
1351				GTGGGCGCCG _CACCCGCGCC	
1401	••••			CAACACCGCC GTTGTGGCGG	
1451				ACGAGGAGGT TGCTCCTCCA	
1501				ACCTACAAGG TGGATGTTCC	
1551				CCTGGAGGGC GGACCTCCCG	
1601	CCCAGAAGAG GGGTCTTCTC				CACCCAGGGC GTGGGTCCCG
1651	TACTTCCCCG ATGAAGGGGC	ACTGGCAGAA TGACCGTCTT	CTACACCCCC	GGCCCCGGCA	TCAGGTTCCC
1701	CCTGACCTTC GGACTGGAAG				CCCGAGAAGG GGGCTCTTCC
1751	TGGAGGAGGC ACCTCCTCCG	CAACGAGGGC GTTGCTCCCG	GAGAACAACT CTCTTGTTGA	GCGCCGCCA CGCGGCGGCT	CCCCATGTCC GGGGTACAGG

Figure 27B

1801					GGAGGT FA CCTCCAAGCT
1851					CCCGAGTACT
	GAGGTTCGAC	CGGAAGGTGG	TGCACCGGTC	CCTCGACGTG	GGGCTCATGA
1901				CTGTGCCTTC	
	TGTTCCTGAC	GATTTCGGGC	CCGTCTAGAC	GACACGGAAG	ATCAACGGTC
1951				TCCTTGACCC	
	GGTAGACAAC	AAACGGGGAG	GGGGCACGGA	AGGAACTGGG	ACCTTCCACG
2001					TCGCATTGTC
	GTGAGGGTGA	CAGGAAAGGA	TTATTTTACT	CCTTTAACGT	AGCGTAACAG
2051					GGACAGCAAG
	ACTCATCCAC	AGTAAGATAA	GACCCCCCAC	CCCACCCCGT	CCTGTCGTTC
2101					CGGTGGGCTC
	CCCCTCCTAA	CCCTTCTGTT	ATCGTCCGTA	CGACCCCTAC	GCCACCCGAG
2151					GCTTAAGGGT
	ATACCGGCTA	GCCGCGCGGC	ATGACTTTAC	ACACCCGCAC	CGAATTCCCA
2201					ATCTGTTTTG
	CCCTTTCTTA	TATATTCCAC	CCCCAGAATA	CATCAAAACA	TAGACAAAAC
2251				CGTTTGATGG	
	GTCGTCGGCG	GCGGCGGTAC	TCGTGGTTGA	GCAAACTACC	TTCGTAACAC
2301			_		TGCGTCAGAA
	TCGAGTATAA	ACTGTTGCGC	GTACGGGGGT	ACCCGGCCCC	ACGCAGTCTT
2351					GCAAACTCTA
	ACACT ACCCG	AGGTCGTAAC	TACCAGCGGG	GCAGGACGGG	CGTTTGAGAT
2401					GACTGCAGCC
	GATGGAACTG	GATGCTCTGG	CACAGACCTT	GCGGCAACCT	CTGACGTCGG
2451			- +		TTGTGACTGA
	AGGCGGCGGC	GAAGTCGGCG	ACGTCGGTGG	CGGGCGCCCT	AACACTGACT
2501					CGTTCATCCG
	GAAACGAAAG	GACTCGGGCG	AACGTTTGTC	ACGTCGAAGG	GCAAGTAGGC
2551	CCCGCGATGA				
	GGGCGCTACT	GTTCAACTGC	CGAGAAAACC	GTGTTAACCT	AAGAAACTGG
2601					GCCAGCAGGT
	GCCCTTGAAT	TACAGCAAAG	AGTCGTCGAC	AACCTAGACG	CGGTCGTCCA
2651					AACATAAATA
	AAGACGGGAC	TTCCGAAGGA	GGGGAGGGTT	ACGCCAAATT	TTGTATTTAT
2701					TTGCTGTCTT
	TTTTTGGTCT	GAGACAAACC	TAAACCTAGT	TCGTTCACAG	AACGACAGAA

Figure 27C

2751				CGGGACCAGC	
2801	GTTGAGGGTC				
				CACCATTTCC	
2851				TGGGGTGGAG ACCCCACCTC	
2901				TAGATGATCC ATCTACTAGG	
2951				TTTCAGTAGC	
	CCTCGCGACC	CGCACCACGG	ATTTTTACAG	AAAGTCATCG	TTCGACTAAC
3001				CAAAGCGGTT GTTTCGCCAA	
3051				TTGGACTGTA AACCTGACAT	
3101				ATTCATGTTG TAAGTACAAC	
3151				ATTTGTCATG TAAACAGTAC	
3201				TTGTGACCTC AACACTGGAG	
3251				CCCACGGGCG GGGTGCCCGC	
3301				AGTTGTGTTC TCAACACAAG	
3351				CGGAGGGTGC GCCTCCCACG	
3401				GTTACCCTCA CAATGGGAGT	
3451				TCATGTCTAC AGTACAGATG	CTGCGGGGCG GACGCCCCGC
3501					AAGAAAGCAG TTCTTTCGTC
3551	GTTCCTGAGC CAAGGACTCG	AGCTGCGACT TCGACGCTGA	TACCGCAGCC ATGGCGTCGG	GGTGGGCCCG CCACCCGGGC	TAAATCACAC ATTTAGTGTG
3601					GCCGTCATCC CGGCAGTAGG
3651					GCATGTTTTC CGTACAAAAG

Figure 270

3701	CCTGACCAAA GGACTGGTTT	ECCAGAA AGGCGGTCTT	GGCGCTCGCC CCGCGAGCGG	GCCCAGCGAT CGGGTCGCTA	AGCAGT TT TCGTCAAGAA
3751	GCAAGGAAGC CGTTCCTTCG		AACGGTTTGA TTGCCAAACT		
3801	CTTTTGAGCG GAAAACTCGC		CAGTTCCAGG GTCAAGGTCC		
3851			CCAGCATATC GGTCGTATAG		
3901	GCGGCTTTCG CGCCGAAAGC		GTAGTCGGTG CATCAGCCAC		
3951			AGGGTCCTCG TCCCAGGAGC		
4001					GCTTGAGGCT CGAACTCCGA
4051			GCTGCCGGTC CGACGGCCAG		GCGTCGGCCA CGCAGCCGGT
4101			TCATAGTCCA AGTATCAGGT		GGCGTGGCCC CCGCACCGGG
4151			GGAGGAGGCG CCTCCTCCGC		GGCAGTGCAG CCGTCACGTC
4201					TCCGGGGAGT AGGCCCCTCA
4251					CACGAGCCAG GTGCTCGGTC
4301					CATGCTTTTT GTACGAAAAA
4351					CGCTCGCTGA GCGAGCCACT
4401					CCTGTCCTCG GGACAGGAGC
4451	AGCGGTGTTC TCGCCACAAG				ACTCTGAGAC TGAGACTCTG
4501					GAGGGGTAGC CTCCCCATCG
4551 .					AAGACACATG TTCTGTGTAC
4601					TGTAGGCCAC ACATCCGGTG

Figure 27E

4651			AAAGGGGGTG TTTCCCCCAC	
4701			CGAGGGCCAG GCTCCCGGTC	
4751	GAGTACTCCC CTCATGAGGG		TCTGCGCTAA AGACGCGATT	
4801			CTGGCCCGCG GACCGGGCGC	_
4851		 	AGACAATCTT TCTGTTAGAA	
4901			TTGGACAGCA AACCTGTCGT	
4951			GGCGCGCTCC CCGCGCGAGG	
5001	TGTTTAGCTG ACAAATCGAC	 	ACCGCCATTC TGGCGGTAAG	
5051			CGCCAACCGC GCGGTTGGCG	
	GGTGACAAGG CCACTGTTCC		_	
5151			AGAATGGCGG CCCCCCCCCCCCCCCCCCCCCCCCCCCCC	TAGGGGGTCT ATCCCCCAGA
5201			ACGGTAAAGA TGCCATTTCT	
5251				TCTAGCGCCT AGATCGCGGA
5301				GAGTGGGGGA CTCACCCCT
5351				CGCAAATGTC GCGTTTACAG
5401				GGGTAGCATC CCCATCGTAG
5451				GTGCGAGGGA CACGCTCCCT
5501				CTGCTCGGAA GACGAGCCTT
5551		 		GTTGGACGCT CAACCTGCGA

Figure 27F

5601	GGAAGACGTT CCTTCTGCAA	CTTCGACCGC	TCTGTGAGAC AGACACTCTG	CTACCGCGTC GATGGCGCAG	ACGCAG TC
5651	GAGGCGTAGG CTCCGCATCC	AGTCGCGCAG TCAGCGCGTC	CTTGTTGACC GAACAACTGG	AGCTCGGCGG TCGAGCCGCC	TGACCTGCAC ACTGGACGTG
5701		CAGTAGTCCA GTCATCAGGT			
5751		TTTCCACAGC AAAGGTGTCG			
5801	TTCCAGTACT AAGGTCATGA	CTTGGATCGG GAACCTAGCC	AAACCCGTCG TTTGGGCAGC	GCCTCCGAAC CGGAGGCTTG	GGTAAGAGCC CCATTCTCGG
5851	TAGCATGTAG ATCGTACATC	AACTGGTTGA TTGACCAACT	CGGCCTGGTA GCCGGACCAT	GGCGCAGCAT CCGCGTCGTA	CCCTTTTCTA GGGAAAAGAT
5901	CGGGTAGCGC GCCCATCGCG	GTATGCCTGC CATACGGACG	GCGGCCTTCC CGCCGGAAGG	GGAGCGAGGT CCTCGCTCCA	GTGGGTGAGC CACCCACTCG
5951	GCAAAGGTGT CGTTTCCACA	CCCTGACCAT GGGACTGGTA	GACTTTGAGG CTGAAACTCC	TACTGGTATT ATGACCATAA	TGAAGTCAGT ACTTCAGTCA
6001		CCGCCCTGCT GGCGGGACGA			CGCTTTTTGG GCGAAAAACC
6051					TATCTTTCCC ATAGAAAGGG
6101					GCACCTCGGA CGTGGAGCCT
6151	TGCCAACAAT	TAATGGACCC	GCCGCTCGTG	CTAGAGCAGT	AAGCCGTTGA TTCGGCAACT
6201					GCCCTTGATG CGGGAACTAC
6251					GGGAGCTGAG CCCTCGACTC
6301	GGGCACGAGA	CTTTCCCGGG	TCAGACGTTC	TACTCCCAAC	GAAGCGACGA CTTCGCTGCT
	TACTCGAGGT	GTCCAGTGCC	CGGTAATCGT	AAACGTCCAC	GTCGCGAAAG CAGCGCTTTC
	CAGGATTTGA	CCGCTGGATA	CCGGTAAAAA	AGACCCCACT	TGCAGTAGAA ACGTCATCTT
	CCATTCGCCC	AGAACAAGGG	TCGCCAGGGT	AGGTTCCAAG	GCGGCTAGGT
6501	CTCGCGCGGC CAGCGCGCCG	AGTCACTAGA TCAGTGATCT	GGCTCATCTC	CGCCGAACTT	CATGACCAGC GTACTGGTCG

Figure 27G

6551	ATGAAGGGCA TACTTCCCGT	GCTCGACGAA	CCCAAAGGCC GGGTTTCCGG	CCCATCCAAG GGGTAGGTTC	TATAGE CONTRACTOR TATACCAGAG
6601				GCGAGGATGC CGCTCCTACG	
6651	GGAAGAACTG	GATCTCCCGC	CACCAATTGG	AGGAGTGGCT	ATTGATGTGG
6701				TCCTCACCGA	
	ACTTTCATCT			GTGAGCACGA	
6751				GGGCTGTACA CCCGACATGT	
6801				AGAGTGGGAA TCTCACCCTT	
6851				ACTTCGGCTG TGAAGCCGAC	
6901			•	GGATCGGACC CCTAGCCTCG	
6951				GCGGTCGGAG CGCCAGCCTC	
7001				TGGAGCTCCC ACCTCGAGGG	
7051	GTCAGGCGGG	AGCTCCTGCA	GGTTTACCTC	GCATAGACGG CGTATCTGCC	GTCAGGGCGC
7101	GGGCTAGATC	CAGGTGATAC	CTAATTTCCA	GGGGCTGGTT	GGTGGCGGCG
7151				CCCCGACCAA	
,131	AGCTACCGAA	CGTTCTCCGG	CGTAGGGGCG	CCGCGCTGAT	GCCATGGCGC
7201				GGATGATGCA CCTACTACGT	TCTAAAAGCG AGATTTTCGC
7251			•	GGGCTCCGGA CCCGAGGCCT	
7301	GAGGGGGCAG CTCCCCCGTC				CTGGTGCTGC GACCACGACG
	GCGCGTAGGT CGCGCATCCA				
7401	CTGGCGCCTC GACCGCGGAG				AACCTGAAAG TTGGACTTTC
7,451	AGAGTTCGAC TCTCAAGCTG				CTGGCGCAAA GACCGCGTTT

Figure 27H

7501	ATCTCCTGCA TAGAGGACGT	ADTOOTS O	GTTGTCTTGA CAACAGAACT	TAGGCGATON ATCCGCTAGA	GCCGGT TT
7551			GGAGATCTCC CCTCTAGAGG		
7601			ATGCGGGCCA TACGCCCGGT		
7651	- · · · - · · · · · · · · · · · · · · ·		GCGGCTĠTAG CGCCGACATC		
7701			GCGCGAGATT CGCGCTCTAA		
7751			CGCTGAAAGA GCGACTTTCT		
7801			GTACATAACC CATGTATTGG		
7851			CAAGGCGCTC GTTCCGCGAG		
7901			GAGTTGCGCG CTCAACGCGC		
7951			GGCGACAGTG CCGCTGTCAC		
8001			CTTCTTCAAT GAAGAAGTTA		
8051			GGCGGTGGGG CCGCCACCCC		ACGGCGGCGA TGCCGCCGCT
8101			GTCGACAAAG CAGCTGTTTC		
8151			TGACGGCGCG ACTGCCGCGC		CGGGGGCGCA GCCCCGCGT
8201			ATGTCCCGGT TACAGGGCCA		CGGGGGGCTG
8251					ATTGTTGTGT TAACAACACA
8301	AGGTACTCCG TCCATGAGGC	CCGCCGAGGG GGCGGCTCCC	ACCTGAGCGA TGGACTCGCT	GTCCGCATCG CAGGCGTAGC	ACCGGATCGG TGGCCTAGCC
8351	AAAACCTCTC TTTTGGAGAG	CAGAAAGGCG CTCTTTCCGC	TCTAACCAGT AGATTGGTCA	CACAGTCGCA GTGTCAGCGT	AGGTAGGCTG TCCATCCGAC
8401	AGCACCGTGG TCGTGGCACC	CGGCCGCCAG GCCGCCGTC	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	TCGGGGTTGT AGCCCCAACA	TTCTGGCGGA AAGACCGCCT

Figure 27I

8451	GGTGCTGCTG CCACGACGAC	TACTACATTA	TAAAGTAGGC ATTTCATCCG	GGTCTTGAGA CCAGAACTCT	0000007ACC
8501		CACCATGTCC GTGGTACAGG			GCGCAGGCGG CGCGTCCGCC
8551		CCCAGGCTTC GGGTCCGAAG			
8601		AGCCTTTCTA TCGGAAAGAT			TCCTCTTGTC AGGAGAACAG
8551		TGCATCTATC ACGTAGATAG			TGGCCGTAGG ACCGGCATCC
8701		TTCCTCCCAT AAGGAGGGTA			TCATCGGCTG AGTAGCCGAC
8751		AGGTCGGCGA TCCAGCCGCT			GCCTGCTGCA CGGACGACGT
8801		GGTAGACTGG CCATCTGACC			GCGGTGGTAT CGCCACCATA
8851					ACCAGTTAAC TGGTCAATTG
8901		CCCGGCTGCG GGGCCGACGC			CGCGAGTAAG GCGCTCATTC
8951					GTACTGGTAT CATGACCATA
9001		AGTGCGGCGG TCACGCCGCC			AGCGTAGGGT TCGCATCCCA
9051		CCGGGGGCGA			TGATATCCGT ACTATAGGCA
9101		GGACATCCAG CCTGTAGGTC			
9151			•		AAAAGTGCTC TTTTCACGAG
9201					TTGACGCTCT AACTGCGAGA
9251					GTGGTCTGGT CACCAGACCA
9301					CGAGCCCCGT GCTCGGGGCA
9351					TGTCGAACCC ACAGCTTGGG

Figure 27J

		_			
9401	AGGTGTGCGA	CARAGACAA	CGGGGGAGTG	CTCCTTTTGG	CTTCCT
. • • • •		GCAGTCTGTT			
9451	פפרפרפפרפפ	CTGCTGCGCT	שלים היים איני איני איני איני איני איני איני א	GCCACTGGCC	GCGCGCAGCG
3431		GACGACGCGA			
	CCGCGCCGCC	GALGACGCGA	ICGAMMANC	CGGIGACCGG	COCOCOTCOC
0501				** CMCCCM20	cmacomom) o
9501		GGCTGGAAAG			
	ATTCGCCAAT	CCGACCTTTC	GCTTTCGTAA	TTCACCGAGC	GAGGGACATC
9551		ATTTTCCAAG			
•	GGCCTCCCAA	TAAAAGGTTC	CCAACTCAGC	GCCCTGGGGG	CCAAGCTCAG
9601	TCGGACCGGC	CGGACTGCGG	CGAACGGGGG	TTTGCCTCCC	CGTCATGCAA
	AGCCTGGCCG	GCCTGACGCC	GCTTGCCCCC	AAACGGAGGG	GCAGTACGTT
9651	GACCCCGCTT	GCAAATTCCT	CCGGAAACAG	GGACGAGCCC	CTTTTTTGCT
	CTGGGGCGAA	CGTTTAAGGA	GCCCTTTCTC	CCTGCTCGGG	GAAAAAACGA
9701	TTTCCCAGAT	GCATCCGGTG	CTGCGGCAGA	TGCGCCCCCC	TCCTCAGCAG
•	AAAGGGTCTA	CGTAGGCCAC	GACGCCGTCT	ACGCGGGGGG	AGGAGTCGTC
			3.1000000		
9751	CCCCAACACC	AAGAGCAGCG	CCACACATCC	AGGGCACCCT	CCCCTCCTCC
3131		TTCTCGTCGC			
	GCCGTTCTCG	TICICGICGC	COICIGIACO	ICCCG1GGGA	GOGGAGGAGG
0001	m> 000000m03	GGAGGGGCGA	CAROCCOCOR	men cececen	CCACAMCCMC
9801				•	
	ATGGCGCAG1	CCTCCCCGCT	GTAGGCGCCA	ACTGCGCCGT	CGTCTACCAC
					CDDCC2 CC2 C
9851		ccccccccc			
	·TAATGCTTGG	GGGCGCCGCG	GCCCGGGCCG	TGATGGACCT	GAACCTCCTC
9901		TGGCGCGGCT			
	CCGCTCCCGG	ACCGCGCCGA	TCCTCGCGGG	AGAGGACTCG	CCGTGGGTTC
9951		AAGCGTGATA			
	CCACGTCGAC	TTCGCACTAT	GCGCACTCCG	CATGCACGGC	GCCGTCTTGG
10001	TGTTTCGCGA	CCGCGAGGGA	GAGGAGCCCG	AGGAGATGCG	GGATCGAAAG
	ACAAAGCGCT	GGCGCTCCCT	CTCCTCGGGC	TCCTCTACGC	CCTAGCTTTC
		· ·			
10051	TTCCACGCAG	GGCGCGAGCT	GCGGCATGGC	CTGAATCGCG	AGCGGTTGCT
		CCGCGCTCGA			
10101	CCCCCACCAC	GACTTTGAGC	CCGACGCGCG	AACCGGGATT	ACTOCOCCOC
10101		CTGAAACTCG			
	CGCGCTCCTC	CIGNMCICG	000100000	11000001721	Tempoococo
30153	00002020	-		CCCCDTDCCD	GCAGACGGTG
10121					
	CGCGTGTGCA	تاقاناتاتاناناتاتات	CIGGACCATT	GGCGTATGCT	CGTCTGCCAC
	*********		8 8 8 8 8 COMM	****	macama acam
10201					TGCGTACGCT
	TTGGTCCTCT	AATIGAAAGT	TITITUGAAA	TIGITIGGIGC	ACGCATGCGA
					macon cress
10251					TGGGACTTTG
	ACACCGCGCG	CTCCTCCACC	GATATCCTGA	CTACGTAGAC	ACCCTGAAAC
		/			
10301					GGCGCAGCTG
	ATTCGCGCGA	CCTCGTTTTG	GGTTTATCGT	TCGGCGAGTA	CCGCGTCGAC

Figure 27K

10351	титесттатас	TGCACAG	. CAGGGACAAC	CACCCATTCA	GGG ATC TT
10331		ACGTCGTGTC			
10401		GTAGAGCCCG CATCTCGGGC			
10451		CATAGTGGTG GTATCACCAC	-		
10501		TCAACTATTC AGTTGATAAG			
10551		CATACCCCTT GTATGGGGAA			
10601		CATGCGCATG GTACGCGTAC			
10651		ATCGCAACGA TAGCGTTGCT			
10701		CTCAGCGACC GAGTCGCTGG			
10751		GGGCAGCGGC CCCGTCGCCG			
10801		TGCGCTGGGC ACGCGACCCG			
10851		GGGCTGGCGG CCCGACCGCC			
10901		ATATGACGAG TATACTGCTC			
10951		TGATGTTTCT ACTACAAAGA			
11001		CGGCGCTGCA GCCGCGACGT			
11051	. CGACTGGCGC GCTGACCGCG	CAGGTCATGG GTCCAGTACC	-		
11101					CGCAATTCTG GCGTTAAGAC
11151	GAAGCGGTGG CTTCGCCACC				AGGTGCTGGC TCCACGACCG
11201		GCGCTGGCCG CGCGACCGGC			GACGAGGCCG CTGCTCCGGC
11251		CGACGCGCTG GCTGCGCGAC			CAACAGCGGC GTTGTCGCCG

Figure 27L

11301	AACGTGCAGA TTGCACGTCT	CCTGGA GGTTGGACCT	CCGGCTGGTG GGCCGACCAC	GGGGATGTGC CCCCTACACG	CCCACC T
11351	GGCGCAGCGT CCGCGTCGCA	GAGCGCGCGC CTCGCGCGCG	AGCAGCAGGG TCGTCGTCCC	CAACCTGGGC GTTGGACCCG	TCCATGGTTG AGGTACCAAC
11401	CACTAAACGC GTGATTTGCG	CTTCCTGAGT GAAGGACTCA	ACACAGCCCG TGTGTCGGGC	CCAACGTGCC GGTTGCACGG	GCGGGGACAG CGCCCTGTC
11451	GAGGACTACA CTCCTGATGT	CCAACTTTGT GGTTGAAACA	GAGCGCACTG CTCGCGTGAC	CGGCTAATGG GCCGATTACC	TGACTGAGAC ACTGACTCTG
11501	ACCGCAAAGT TGGCGTTTCA	GAGGTGTACC CTCCACATGG	AGTCTGGGCC TCAGACCCGG	AGACTATTTT TCTGATAAAA	TTCCAGACCA AAGGTCTGGT
11551		GGACGTCTGG	CATTTGGACT	CGGTCCGAAA	GTTTTTGAAC
11601	GTCCCCGACA	CCCCCACGC	CCGAGGGTGT	GGCGACCGCG CCGCTGGCGC	GCTGGCACAG
11651	ATCGAACGAC	TGCGGGTTGA	GCGCGGACAA	GCTGCTGCTA CGACGACGAT	TATCGCGGGA
11701	AGTGCCTGTC	ACCGTCGCAC	AGGGCCCTGT	CATACCTAGG GTATGGATCC	AGTGAACGAC
11751	TGTGACATGG	CGCTCCGGTA	TCCAGTCCGC	CATGTGGACG GTACACCTGC	TCGTATGAAA
11801	GGTCCTCTAA	TGTTCACAGT	CGGCGCGCGA	GGGGCAGGAG CCCCGTCCTC	CTGTGCCCGT
11851	CGGACCTCCG	TTGGGATTTG	ATGGACGACT	CCAACCGGCG GGTTGGCCGC	CGTCTTCTAG
11901	GGGAGCAACG	TGTCAAATTT	GTCGCTCCTC	GAGCGCATTT CTCGCGTAAA	ACGCGATGCA
11951	CGTCGTCTCG	CACTCGGAAT	TGGACTACGC	CGACGGGGTA GCTGCCCCAT	TGCGGGTCGC
12001	ACCGCGACCT	GTACTGGCGC	GCGTTGTACC	TTGGCCCGTA	GTATGCCTCA CATACGGAGT
	TTGGCCGGCA	AATAGTTGGC	GGATTACCTG	ATGAACGTAG	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
	GCACTTGGGG	CTCATAAAGT	GGTTACGGTA	GAACTTGGGC	CACTGGCTAC GTGACCGATG
12151	CGCCCCTGG	TTTCTACACC	GGGGGATTCG	AGGTGCCCGA TCCACGGGCT	GGGTAACGAT
12201	GGATTCCTCT CCTAAGGAGA	GGGACGACAT CCCTGCTGTA	AGACGACAGC TCTGCTGTCG	GTGTTTTCCC CACAAAAGGG	CGCAACCGCA

Figure 27 M

12251	GACCCTGCTA	G***TTGCAAC	AGCGCGAGCA	GGCAGAGGCG"	GCGC TCZ A A
				CCGTCTCCGC	
	CIGGGACGII	C.SenicG11G	1000001001	CCG1C1CCGC	COCONCO
					•
12301	AGGAAAGCTT	CCGCAGGCCA	AGCAGCTIGT	CCGATCTAGG	CGCTGCGGCC
	TCCTTTCGAA	GGCGTCCGGT	TCGTCGAACA	GGCTAGATCC	GCGACGCCGG

12351	CCCCCCCCAC	ስጥርር ጥ እርጥ እ ር	CCCSTMINICCS	AGCTTGATAG	COMODOMAN
12331					
	GGCGCCAGTC	TACGATCATC	GGGTAAAGGT	TCGAACTATC	CCAGAGAATG
12401	CAGCACTCGC	ACCACCCGCC	CGCGCCTGCT	GGGCGAGGAG	GAGTACCTAA
	GTCGTGAGCG	TECTECECCE	GCGCGGACGA	CCCGCTCCTC	ריזיר איזיכה איזייזי
					010111001111
22452	3.03.3.0moccom				
12451				AAAACCTGCC	
	TGTTGAGCGA	CGACGTCGGC	GTCGCGCTTT	TTTTGGACGG	AGGCCGTAAA
				•	
12501	CCCAACAACG	GGATAGAGAG	CCTACTCCAC	AAGATGAGTA	GATGGAAGAC
				TTCTACTCAT	
	6661161160	CCIMICICIC	GGATCACCTG	IICIACICAI	CIACCIICIG
12551	GTACGCGCAG	GAGCACAGGG	ACGTGCCAGG	CCCGCGCCCG	CCCACCCGTC
	CATGCGCGTC	CTCGTGTCCC	TGCACGGTCC	GGGCGCGGC	GGGTGGGCAG
12601	CTCABACCCA	CCACCCTCAC	CCCCCTCTCC	TGTGGGAGGA	CCATCACTCC
12001					
	CAGTTTCCGT	GCTGGCAGTC	GCCCCAGACC	ACACCCTCCT	GCTACTGAGC
12651	GCAGACGACA	GCAGCGTCCT	GGATTTGGGA	GGGAGTGGCA	ACCCGTTTGC
	CGTCTGCTGT	CGTCGCAGGA	CCTAAACCCT	CCCTCACCGT	TGGGCAAACG
12701	CCACCODOCCC	CCC x CCCmcc	CCNCNNDCDD	AAAAAAATT	2222200200
12/01					
	CGTGGAAGCG	GGGTCCGACC	CCTCTTACAA	AATTTTTTT	TTTTTCGTAC
12751	ATGCAAAATA	AAAAACTCAC	CAAGGCCATG	GCACCGAGCG	TTGGTTTTCT
	TACGTTTTAT	TTTTTGAGTG	GTTCCGGTAC	CGTGGCTCGC	AACCAAAAGA
				0010001000	
7.0007	moma mmonoo	mm> cm> mccc			
12801				ATGTATGAGG	
	ACATAAGGGG	AATCATACGC	CGCGCGCCGC	TACATACTCC	TTCCAGGAGG
12851	TCCCTCCTAC	GAGAGTGTGG	TGAGCGCGGC	GCCAGTGGCG	GCGGCGCTGG
	ACCCACCATO	כיזיכיזיכי אראכיכ	ACTOCCOCCC	CGGTCACCGC	CCCCCCCACC
	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	0101010100	1101000000	COOTENCEOE	COCCOCOACC
12901				CGTTTGTGCC	
	CAAGAGGGAA	GCTACGAGGG	GACCTGGGCG	GCAAACACGG	AGGCGCCATG
12951	CTGCGGCCTA	CCGGGGGGAG	AAACAGCATC	CGTTACTCTG	AGTTGGCACC
				GCAATGAGAC	
	GACGCCGGA1	GGCCCCCTC	IIIGICGIAG	GCMATGAGAC	TCAACCGTGG
13001	CCTATTCGAC	ACCACCCGTG	TGTACCTGGT	GGACAACAAG	TCAACGGATG
	GGATAAGCTG	TGGTGGGCAC	ACATGGACCA	CCTGTTGTTC	AGTTGCCTAC
13051	TGGCATCCCT	CADCTACCAC	AACCACCACA	CC 2 2 COMMON	GACCACGGGC
10001					
	ACCUTAGGGA	CTTGATGGTC	TIGUTGGTGT	CGTTGAAAGA	CTGGTGCCAG
13101	ATTCAAAACA	ATGACTACAG	CCCGGGGGAG	GCAAGCACAC	AGACCATCAA
	TAAGTTTTGT	TACTGATGTC	GGGCCCCCTC	CGTTCGTGTG	TCTGGTAGTT
12151	TCTTGACGAC	CCCDCC V Cm	CCCCCCCC	CCMCNNNNCC	プロプレイルへい かい x
13131					
	AGAACTGCTG	GCCAGCGTGA	CCCCGCCGCT	GGACTTTTGG	TAGGACGTAT

Figure 27N

			•		
13201	CCAACATGCC GGTTGTACGG	ALTGTGAAC TACACTTG	GAGTTCATGT CTCAAGTACA	TTACCAATAA ~ AATGGTTATT	CAAATT C
13251	CGGGTGATGG GCCCACTACC	TGTCGCGCTT ACAGCGCGAA	GCCTACTAAG CGGATGATTC	GACAATCAGG CTGTTAGTCC	TGGAGCTGAA ACCTCGACTT
13301	ATACGAGTGG	GTGGAGTTCA	CGCTGCCCGA	GGGCAACTAC CCCGTTGATG	TCCGAGACCA
13351	TGACCATAGA ACTGGTATCT	CCTTATGAAC GGAATACTTG	AACGCGATCG TTGCGCTAGC	TGGAGCACTA ACCTCGTGAT	CTTGAAAGTG GAACTTTCAC
13401	GGCAGACAGA	ACGGGGTTCT	GGAAAGCGAC	ATCGGGGTAA	AGTTTGACAC
	CCGTCTGTCT	TGCCCCAAGA	CCTTTCGCTG	TAGCCCCATT	TCAAACTGTG
13451	CCGCAACTTC GCCGTTGAAG	AGACTGGGGT TCTGACCCCA	TTGACCCCGT AACTGGGGCA	CACTGGTCTT GTGACCAGAA	GTCATGCCTG CAGTACGGAC
13501	GGGTATATAC	AAACGAAGCC	TTCCATCCAG	ACATCATTTT	GCTGCCAGGA
	CCCATATATG	TTTGCTTCGG	AAGGTAGGTC	TGTAGTAAAA	CGACGGTCCT
13551	TGCGGGGTGG	ACTTCACCCA	CAGCCGCCTG	AGCAACTTGT	TGGGCATCCG
	ACGCCCCACC	TGAAGTGGGT	GTCGGCGGAC	TCGTTGAACA	ACCCGTAGGC
13601	CAAGCGGCAA	CCCTTCCAGG	AGGGCTTTAG	GATCACCTAC	GATGATCTGG
	GTTCGCCGTT	GGGAAGGTCC	TCCCGAAATC	CTACTGGATG	CTACTAGACC
13651	AGGGTGGTAA	CATTCCCGCA	CTGTTGGATG	TGGACGCCTA	CCAGGCGAGC
	TCCCACCATT	GTAAGGGCGT	GACAACCTAC	ACCTGCGGAT	GGTCCGCTCG
13701	TTGAAAGATG	ACACCGAACA	GGGCGGGGGT	GGCGCAGGCG	GCAGCAACAG
	AACTTTCTAC	TGTGGCTTGT	CCCGCCCCCA	CCGCGTCCGC	CGTCGTTGTC
13751	CAGTGGCAGC	GGCGCGGAAG	AGAACTCCAA	CGCGGCAGCC	GCGGCAATGC
	GTCACCGTCG	CCGCGCCTTC	TCTTGAGGTT	GCGCCGTCGG	CGCCGTTACG
13801				TTCGCGGCGA	
	TCGGCCACCT	CCTGTACTTG	CTAGTACGGT	AAGCGCCGCT	GTGGAAACGG
13851	ACACGGGCTG	AGGAGAAGCG	CGCTGAGGCC	GAAGCAGCGG	CCGAAGCTGC
	TGTGCCCGAC	TCCTCTTCGC	GCGACTCCGG	CTTCGTCGCC	GGCTTCGACG
13901	CGCCCCGCT GCGGGGGGA	GCGCAACCCG CGCGTTGGGC	AGGTCGAGAA TCCAGCTCTT	GCCTCAGAAG CGGAGTCTTC	AAACCGGTGA TTTGGCCACT
13951	TCAAACCCCT	GACAGAGGAC	AGCAAGAAAC	GCAGTTACAA	CCTAATAAGC
	AGTTTGGGGA	CTGTCTCCTG	TCGTTCTTTG	CGTCAATGTT	GGATTATTCG
14001	AATGACAGCA	CCTTCACCCA	GTACCGCAGC	TGGTACCTTG	CATACAACTA
	TTACTGTCGT	GGAAGTGGGT	CATGGCGTCG	ACCATGGAAC	GTATGTTGAT
14051	CGGCGACCCT	CAGACCGGAA	TCCGCTCATG	GACCCTGCTT	TGCACTCCTG
	GCCGCTGGGA	GTCTGGCCTT	AGGCGAGTAC	CTGGGACGAA	ACGTGAGGAC
14101	ACGTAACCTG TGCATTGGAC	CGGCTCGGAG	CAGGTCTACT	GGTCGTTGCC CCAGCAACGG	AGACATGATG TCTGTACTAC

Figure 270

14151	CAAGACCCCG CTTCTGGGGC	TYTTCCG ACTGGAAGGC	CTCCACGCGC GAGGTGCGCG	CAGATCAGCA GTCTAGTCGT	ACTTTC T TGAAAGGCCA
14201		GAGCTGTTGC CTCGACAACG			
14251	AGGCCGTCTA TCCGGCAGAT	CTCCCAACTC CAGGGTTCAG	ATCCGCCAGT TAGGCGCTCA	TTACCTCTCT AATGGAGAGA	GACCCACGTG CTGGGTGCAC
14301		TTCCCGAGAA AAGGGCTCTT			
14351		GTCAGTGAAA CAGTCACTTT			
14401		CAACAGCATC GTTGTCGTAG			
14451		GCACCTGCCC CGTGGACGGG			
14501		CTATCGAGCC GATAGCTCGG			
14551		CAATAACACA GTTATTGTGT			
14501		CCAAGAAGCG GGTTCTTCGC			
14651		GCGCCCTGGG			
14701		TGACGCCATC ACTGCGGTAG			
14751		CGCCACCAGT GCGGTGGTCA			
14801		GCCCGGCGCT CGGGCCGCGA			
14851		CCACCGCCGC GGTGGCGGCG			
14901		TTAACCGCGC AATTGGCGCG			
14951	CCCCCTCGA CCGGCGAGCT	AGGCTGGCCG TCCGACCGGC			
15001	GGCGACGAGC CCGCTGCTCG	GGCCGCCGCA CCGGCGCGT			
15051	GGTCGCAGGG CCAGCGTCCC				GCGGCCTGCG CGCCGGACGC

Figure 27P

15101	CGTGCCCGTG	ecercecc creccecc	CCCCGCGCAA GGGGCGCGTT	CTAGATTGCA GATCTAACGT	TCTTTTT A
15151				GCCGCCGCCGC	
15201				ATGCTCCAGG TACGAGGTCC	
15251 ·	GGAGATCTAT CCTCTAGATA			GCAGGATTAC CGTCCTAATG	
15301				ATGATGATGA TACTACTACT	
15351				CCCAGGCGAC GGGTCCGCTG	
15401	GAAAGGTCGA CTTTCCAGCT				ACCGTAGTCT TGGCATCAGA
15451					GTATGATGAG CATACTACTC
15501					GCCTCGGGGA CGGAGCCCCT
15551					CCGCTGGACG GGCGACCTGC
15601					GCAGGTGCTG CGTCCACGAC
15651	CCCGCGCTTG GGGCGCGAAC	CACCGTCCGA GTGGCAGGCT	AGAAAAGCGC TCTTTTCGCG	GGCCTAAAGC CCGGATTTCG	GCGAGTCTGG CGCTCAGACC
15701					CAGCGACTGG GTCGCTGACC
15751	AAGATGTCTT TTCTACAGAA	GGAAAAAATG CCTTTTTTAC	ACCGTGGAAC TGCCACCTTG	CTGGGCTGGA GACCCGACCT	GCCCGAGGTC CGGGCTCCAG
15801					TGCAGACCGT ACGTCTGGCA
15851					ACCGCCACAG TGGCGGTGTC
15901	AGGGCATGGA TCCCGTACCT	GACACAAACG CTGTGTTTGC	TCCCCGCTTG AGGGGCCAAC	CCTCAGCGGT GGAGTCGCCA	CCCCCTACCC
15951	ecgetgeagg cgccacgtcc	CGGTCGCTGC GCCAGCGACG	GCCGCGTCC	AAGACCTCTA .TTCTGGAGAT	CGGAGGTGCA
16001					CCGCGCCGTT

Figure 270

16051		eccececce eccececce		_
16101		CGCCTACCCC GCGGATGGGG	 	
16151		ACTACCCGAC TGATGGGCTG	 	cecececece .
16201 .	•	CCAGCCCGTG GGTCGGGCAC	 	
16251		GCAGGACCCT CGTCCTGGGA	 	
16301		AAGCCGGTCT TTCGGCCAGA	 	
16351		TTTCCCGGTG AAAGGGCCAC		
16401		CCGGCCACGG GGCCGGTGCC		
16451	•	CGCGCGTCGC GCGCGCAGCG	 	
16501		ACTGATCGCC TGACTAGCGG		
16551		TGCAGGCGCA ACGTCCGCGT		GTTGCATGTG CAACGTACAC
16601		AATAAAAAGT TTATTTTCA	 	GGTCCTGTAA CCAGGACATT
16651	*	GAATGGAAGA CTTACCTTCT	 	CCCCGCGACA GGGGCGCTGT
16701		CCGTTCATGG GGCAAGTACC	 	ACCAGCAATA TGGTCGTTAT
16751				CATTAAAAAT GTAATTTTTA
16801				ACAGCAGCAC TGTCGTCGTG
16851				CAACAAAAGG GTTGTTTTCC
16901				CCTGGCCAAC GGACCGGTTG
16951				GCCCTCCCGT CGGGAGGGCA

Figure 27R

17001		cereccecc carcecce.			
17051		CCCCCCGAC			
17101	GAGCCTCCCT CTCGGAGGGA	CCTACGAGGA GCATGCTCCT	GGCACTAAAG CCGTGATTTC	CAAGGCCTGC GTTCCGGACG	CCACCACCCG GGTGGTGGGC
17151		CCCATGGCTA GGGTACCGAT			
17201		GCCTCCCCCC			
17251		CCGTTGTTGT			
17301		GGTCCGCGAT CCAGGCGCTA			
17351		GAACAGCATC CTTGTCGTAG			
17401		TCTGATAGCT AGACTATCGA			
17451		CCAGAGGAGC GGTCTCCTCG			
17501		CTTCGATGAT GAAGCTACTA			
17551		TCGGAGTACC AGCCTCATGG			
17601		GTACTTCAGC CATGAAGTCG			
17651		ACGACGTGAC TGCTGCACTG			
17701		GTGGACCGTG CACCTGGCAC			
17751		TGTGGGTGAT ACACCCACTA			
17801					AGCCCTACTC TCGGGATGAG
17851					AATCCTTGCG TTAGGAACGC
17901	AATGGGATGA TTACCCTACT	AGCTGCTACT TCGACGATGA	GCTCTTGAAA CGAGAACTTT	TAAACCTAGA ATTTGGATCT	AGAAGAGGAC TCTTCTCCTG

Figure 275

17951			GCTGAGCAGC CGACTCGTCG	
18001			 AAATATTACA TTTATAATGT	
18051	TTCAAATAGG AAGTTTATCC		 AATATGCCGA TTATACGGCT	
18101		·	 TGGTACGAAA ACCATGCTTT	
18151	TCATGCAGCT AGTACGTCGA		TACCCCAATG ATGGGGTTAC	
18201			 ATGGAGGGCA TACCTCCCGT	
18251			CAAGTGGAAA GTTCACCTTT	
18301	•		 TGATAACTTG ACTATTGAAC	
18351			 AAACCCCAGA TTTGGGGTCT	
18401			TCACGAGAAC AGTGCTCTTG	
18451			TGCTTTTAGG ACGAAAATCC	GACAATTTTA CTGTTAAAAT
18501			ATATGGGTGT TATACCCACA	
18551			TTGCAAGACA AACGTTCTGT	GAAACACAGA CTTTGTGTCT
18601			TGGTGATAGA ACCACTATCT	
18651			 ATGATCCAGA TACTAGGTCT	
18701				GCTTTCCACT CGAAAGGTGA
18751	CCCTCCACAC			CCTAAAACAG GGATTTTGTC
18801	GTCAGGAAAA CAGTCCTTTT			AGATAAAAAT TCTATTTTTA
18851				TAAATGCCAA ATTTACGGTT

Figure 27T

18901	CCTGTGGAGA GGACACCTCT				
18951	AGCTAAAGTA TCGATTTCAT	CAGTCCTTCC GTCAGGAAGG			
19001		TGAACAAGCG ACTTGTTCGC			
19051	CATTAACCTT GTAATTGGAA	GGAGCACGCT CCTCGTGCGA			
19101		CCACCGCAAT GGTGGCGTTA			
19151		GCTATGTGCC CGATACACGG			
19201		AACCTCCTTC TTGGAGGAAG			
19251		GGATGTTAAC CCTACAATTG			
19301	CTAAGGGTTG GATTCCCAAC	ACGGAGCCAG TGCCTCGGTC	CATTAAGTTT GTAATTCAAA	GATAGCATTT CTATCGTAAA	GCCTTTACGC CGGAAATGCG
19351	CACCTTCTTC GTGGAAGAAG	CCCATGGCCC GGGTACCGGG	ACAACACCGC TGTTGTGGCG	CTCCACGCTT GAGGTGCGAA	GAGGCCATGC CTCCGGTACG
19401	TTAGAAACGA AATCTTTGCT	CACCAACGAC GTGGTTGCTG	CAGTCCTTTA GTCAGGAAAT	ACGACTATCT TGCTGATAGA	CTCCGCCGCC GAGGCGGCGG
19451	AACATGCTCT TTGTACGAGA	ACCCTATACC TGGGATATGG	CGCCAACGCT GCGGTTGCGA	ACCAACGTGC TGGTTGCACG	CCATATCCAT GGTATAGGTA
19501	CCCCTCCCGC	AACTGGGCGG TTGACCCGCC	CTTTCCGCGG GAAAGGCGCC	CTGGGCCTTC GACCCGGAAG	ACGCGCCTTA TGCGCGGAAT
19551.	AGACTAAGGA TCTGATTCCT	AACCCCATCA TTGGGGTAGT	CTGGGCTCGG GACCCGAGCC	GCTACGACCC CGATGCTGGG	TTATTACACC AATAATGTGG
19601	TACTCTGGCT ATGAGACCGA	CTATACCCTA GATATGGGAT	CCTAGATGGA GGATCTACCT	ACCTTTTACC TGGAAAATGG	TCAACCACAC AGTTGGTGTG
19651	CTTTAAGAAG GAAATTCTTC	GTGGCCATTA CACCGGTAAT	CCTTTGACTC GGAAACTGAG	TTCTGTCAGC AAGACAGTCG	TGGCCTGGCA ACCGGACCGT
19701	ATGACCGCCT TACTGGCGGA	GCTTACCCCC	AACGAGTTTG TTGCTCAAAC	AAATTAAGCG TTTAATTCGC	CTCAGTTGAC GAGTCAACTG
19751	GGGGAGGGTT CCCCTCCCAA	ACAACGTTGC TGTTGCAACG	CCAGTGTAAC GGTCACATTG	ATGACCAAAG TÄCTGGTTTC	ACTGGTTCCT TGACCAAGGA
19801	GGTACAAATG CCATGTTTAC	CTAGCTAACT GATCGATTGA	ATAACATTGG TATTGTAACC	CTACCAGGGC	TTCTATATCC AAGATATAGG

Figure 274

19851	CAGAGAGCTA GTCTCTCGAT	CL GACCGC GTTCCTGGCG	ATGTACTCCT TACATGAGGA	TCTTTAGAAA AGAAATCTTT	CTTCCA(CCC GAAGGTCGGG
19901	ATGAGCCGTC TACTCGGCAG	AGGTGGTGGA TCCACCACCT	TGATACTAAA ACTATGATTT	TACAAGGACT ATGTTCCTGA	ACCAACAGGT TGGTTGTCCA
19951	GGGCATCCTA CCCGTAGGAT	CACCAACACA GIGGTIGTGT	ACAACTCTGG TGTTGAGACC	ATTTGTTGGC TAAACAACCG	TACCTTGCCC ATGGAACGGG
- 20001	CCACCATGCG GGTGGTACGC	CGAAGGACAG GCTTCCTGTC	GCCTACCCTG CGGATGGGAC	CTAACTTCCC GATTGAAGGG	CTATCCGCTT GATAGGCGAA
20051	ATAGGCAAGA TATCCGTTCT	CCGCAGTTGA GGCGTCAACT	CAGCATTACC GTCGTAATGG	CAGAAAAAGT GTCTTTTCA	TTCTTTGCGA AAGAAACGCT
20101	TCGCACCCTT AGCGTGGGAA	TGGCGCATCC ACCGCGTAGG	CATTCTCCAG GTAAGAGGTC	TAACTTTATG ATTGAAATAC	TCCATGGGCG AGGTACCCGC
20151	CACTCACAGA GTGAGTGTCT	CCTGGGCCAA GGACCCGGTT	AACCTTCTCT TTGGAAGAGA	ACGCCAACTC TGCGGTTGAG	CGCCCACGCG GCGGGTGCGC
20201	CTAGACATGA GATCTGTACT	CTTTTGAGGT GAAAACTCCA	GGATCCCATG CCTAGGGTAC	GACGAGCCCA CTGCTCGGGT	CCCTTCTTTA GGGAAGAAAT
20251		GAAGTCTTTG CTTCAGAAAC			
20301		AACCGTGTAC TTGGCACATG			
20351	ACAACATAAA TGTTGTATTT	GAAGCAAGCA CTTCGTTCGT	ACATCAACAA TGTAGTTGTT	CAGCTGCCGC GTCGACGGCG	CATGGGCTCC GTACCCGAGG
20401		AACTGAAAGC TTGACTTTCG			
20451		ACCTATGACA TGGATACTGT			
20501		CGCCATAGTC GCGGTATCAG			
20551		CCTTTGCCTG GGAAACGGAC			
20601		GGCTTTTCTG CCGAAAAGAC			
20651	AGTACGAGTC TCATGCTCAG	ACTCCTGCGC TGAGGACGCG			
20701	TGTATAACGC ACATATTGCG	TGGAAAAGTC ACCTTTTCAG			
20751	CGCCTGTGGA GCGGACACCT				

Figure 27 V.

20801	CCCAAACTCC GGGTTTGAGG	GATCAC GTACCTAGTG	AACCCCACCA TTGGGGTGGT	TGAACCTTAT ACTTGGAATA	TACCGG A
20851		TGCTCAACAG ACGAGTTGTC			
20901		CTCTACAGCT GAGATGTCGA			
20951		GCAGATTAGG CGTCTAATCC			
21001		AATGTACTAG TTACATGATC			
21051	TTTGTACACT	CTCGGGTGAT	TATTTACCCC	CACCCTTGCC	GTCTGCGCCG
	AAACATGTGA	GAGCCCACTA	ATAAATGGGG	GTGGGAACGG	CAGACGCGGC
21101	TTTAAAAATC	AAAGGGGTTC	TGCCGCGCAT	CGCTATGCGC	CACTGGCAGG
	AAATTTTTAG	TTTCCCCAAG	ACGGCGCGTA	GCGATACGCG	GTGACCGTCC
21151	GACACGTTGC	GATACTGGTG	TTTAGTGCTC	CACTTAAACT	CAGGCACAAC
	CTGTGCAACG	CTATGACCAC	AAATCACGAG	GTGAATTTGA	GTCCGTGTTG
21201	CATCCGCGGC	AGCTCGGTGA	AGTTTTCACT	CCACAGGCTG	CGCACCATCA
	GTAGGCGCCG	TCGAGCCACT	TCAAAAGTGA	GGTGTCCGAC	GCGTGGTAGT
21251	CCAACGCGTT	TAGCAGGTCG	GGCGCCGATA	TCTTGAAGTC	GCAGTTGGGG
	GGTTGCGCAA	ATCGTCCAGC	CCGCGGCTAT	AGAACTTCAG	CGTCAACCCC
21301	CCTCCGCCCT	GCGCGCGCGA	GTTGCGATAC	ACAGGGTTGC	AGCACTGGAA
	GGAGGCGGGA	CGCGCGCGCT	CAACGCTATG	TGTCCCAACG	TCGTGACCTT
21351	CACTATCAGC	GCCGGGTGGT	GCACGCTGGC	CAGCACGCTC	TTGTCGGAGA
	GTGATAGTCG	CGGCCCACCA	CGTGCGACCG	GTCGTGCGAG	AACAGCCTCT
21401	TCAGATCCGC	GTCCAGGTCC	TCCGCGTTGC	TCAGGGCGAA	CGGAGTCAAC
	AGTCTAGGCG	CAGGTCCAGG	AGGCGCAACG	AGTCCCGCTT	GCCTCAGTTG
21451	TTTGGTAGCT	CCCTTCCCAA	AAAGGGCGCG	TGCCCAGGCT	TTGAGTTGCA
	AAACCATCGA	CGGAAGGGTT	TTTCCCGCGC	ACGGGTCCGA	AACTCAACGT
21501	CTCGCACCGT	AGTGGCATCA	AAAGGTGACC	GTGCCCGGTC	TGGGCGTTAG
	GAGCGTGGCA	TCACCGTAGT	TTTCCACTGG	CACGGGCCAG	ACCCGCAATC
21551	GATACAGCGC	CTGCATAAAA	GCCTTGATCT	GCTTAAAAGC	CACCTGAGCC
	CTATGTCGCG	GACGTATTTT	CGGAACTAGA	CGAATTTTCG	GTGGACTCGG
21601	TTTGCGCCTT	CAGAGAAGAA	CATGCCGCAA	GACTTGCCGG	AAAACTGATT
	AAACGCGGAA	GTCTCTTCTT	GTACGGCGTT	CTGAACGGCC	TTTTGACTAA
21651	GGCCGGACAG	GCCGCGTCGT	GCACGCAGCA	CCTTGCGTCG	GTGTTGGAGA
	CCGGCCTGTC	CGGCGCAGCA	CGTGCGTCGT	GGAACGCAGC	CACAACCTCT
21701	TCTGCACCAC	ATTTCGGCCC	CACCGGTTCT	TCACGATCTT	GGCCTTGCTA
	AGACGTGGTG	TAAAGCCGGG	GTGGCCAAGA	AGTGCTAGAA	CCGGAACGAT

Figure 27 W

21751				TCGCTCGTCA AGCGAGCAGT	
21801 .	•			TCCGTGTAGA AGGCACATCT	
21851				ACAACGCGCA TGTTGCGCGT	
21901				GACTGCAGGT CTGACGTCCA	
21951				GTTGCTGGTG CAACGACCAC	
22001				TCTTGCATAC AGAACGTATG	
22051			-	TTCGCCTTTA AAGCGGAAAT	
22101				AGCCTCCATG TCGGAGGTAC	
22151				TCATCACCGT AGTAGTGGCA	
22201				TGCGTCCGCA ACGCAGGCGT	
22251				TGTGCGCTTA ACACGCGAAT	
22301				AACCCACCAT TTGGGTGGTA	
22351				ATTACCTCTG TAATGGAGAC	
22401				TTTCTTCTTG AAAGAAGAAC	
22451		• •		GGCTGGGTGT CCGACCCACA	
22501					TACGCCGCCT ATGCGGCGGA
22551					GGGGACGGGG
22601				GCGCCGCACC CGCGGCGTGG	GCGTCCGCGC CGCAGGCGCG
22651					TTTCCTTCTC AAAGGAAGAG

Figure 27X

22701	CTATAGGCAG	AGATCA	TGGAGTCAGT	CGAGAAGAAG	GACAGC
	GATATCCGTC	TTTTTCTAGT	ACCTCAGTCA	GCTCTTCTTC	CTGTCGGATT
22751		TGÄGTTCGCC ACTCAAGCGG			
22801		TCCCCGTCGA AGGGGCAGCT			
22851	TATCGAGCAG	GACCCAGGTT	TTGTAAGCGA	AGACGACGAG	GACCGCTCAG
22901		CTGGGTCCAA GGATAAAAAG			
22901		CCTATTTTTC			
22951		GGCGGGGGGA CCGCCCCCT			
23001		CTGTTGAAGC GACAACTTCG			
23051	ACGCGTTGCA	AGAGCGCAGC TCTCGCGTCG	GATGTGCCCC	TCGCCATAGC	GGATGTCAGC
23101		AACGCCACCT			
23101	GAACGGATGC	TTGCGGTGGA	TAAGAGTGGC	GCGCATGGGG	GGTTTGCGGT
23151	AGAAAACGGC TCTTTTGCCG	ACATGCGAGC TGTACGCTCG	CCAACCCGCG GGTTGGGCGC	CCTCAACTTC GGAGTTGAAG	TACCCCGTAT ATGGGGCATA
23201		AGAGGTGCTT TCTCCACGAA			CCAAAACTGC GGTTTTGACG
23251					ACAAGCAGCT TGTTCGTCGA
23301					CTCAACGAAG GAGTTGCTTC
23351	TGCCAAAAAT	CTTTGAGGGT	CTTGGACGCG	ACGAGAAGCG	CGCGGCAAAC
23401					GAGTGTTGGT
	CGAGACGTTG	TCCTTTTGTC	GCTTTTACTT	TCAGTGAGAC	CTCACAACCA
23451	GGAACTCGAG CCTTGAGCTC	GGTGACAACG	CGCGCCTAGC	CGTACTAAAA GCATGATTTT	CGCAGCATCG
23501	AGGTCACCCA TCCAGTGGGT	CTTTGCCTAC GAAACGGATG	CCGGCACTTA GGCCGTGAAT	ACCTACCCCC TGGATGGGGG	CAAGGTCATG GTTCCAGTAC
23551	AGCACAGTCA TCGTGTCAGT	TGAGTGAGCT ACTCACTCGA	GATCGTGCGC CTAGCACGCG	CGTGCGCAGC GCACGCGTCG	CCCTGGAGAG GGGACCTCTC
23601	GGATGCAAAT CCTACGTTTA	TTGCAAGAAC AACGTTCTTG	AAACAGAGGA TTTGTCTCCT	GGGCCTACCC CCCGGATGGG	GCAGTTGGCG GCTCAACCGC

Figure 27 Y

23651	ACGAGCAGCI	· A CCCTCC	፡		CGACTT
20022	TGCTCGTCG	TCGCGCGAC	GAAGTTTGC	CONGCCIGO CONTROL	GCTGAACCTC
23701	GAGCGACGCA	AACTAATGAT	GGCCGCAGTG	CTCGTTACC	TGGAGCTTGA
	CTCGCTGCGT	TTGATTACTA	CCGGCGTCAC	GAGCAATGG	ACCTCGAACT
23751	GTGCATGCAG	CCCTAINCAINANC	י כשכי בככככי	C)#C()	AAGCTAGAGG
23.31	CACGTACGTC	GCCAAGAAAC	CIGACCCGGA	CATGCAGCGC	: AAGCTAGAGG
			· Gacioogcci	. cincipicaco	FITCGATCTCC
23801	AAACATTGCA	CTACACCTTT	CGACAGGGCT	ACGTACGCCA	GGCCTGCAAG
	TTTGTAACGT	GATGTGGAAA	GCTGTCCCGA	TGCATGCGGT	CCGGACGTTC
23851					
23031	TACACCARUG	TGGAGCTCTG	CAACCTGGTC	TCCTACCTTG	GAATTTTGCA
	IAGAGGIIGC	ACCICGAGAC	GTTGGACCAG	AGGATGGAAC	CTTAAAACGT
23901	CGAAAACCGC	CTTGGGCAAA	ACGTGCTTCA	TTCCACGCTC	AAGGGCGAGG
	GCTTTTGGCG	GAACCCGTTT	TGCACGAAGT	AAGGTGCGAG	TTCCCGCTCC
23951	CGCGCCGCGA	CTACGTCCGC	GACTGCGTTT	ACTTATTTCT	ATGCTACACC
	GUGUGUGUT	GATGCAGGCG	CTGACGCAAA	TGAATAAAGA	TACGATGTGG
24001	TGGCAGACGG	CCATGGGCGT	ምተር ርር ልር ርር ልር	- ጥ ርርጥጥርር አርር	AGTGCAACCT
	ACCGTCTGCC	GGTACCCGCA	AACCGTCGTC	ACGAACCTCC	TCACGTTGGA
24051	CAAGGAGCTG	CAGAAACTGC	TAAAGCAAAA	CTTGAAGGAC	CTATGGACGG
	GTTCCTCGAC	GTCTTTGACG	ATTTCGTTTT	GAACTTCCTG	GATACCTGCC
24101	ССТТСВВССВ	CCCCTCCCTC	CCCCCCCAACC	mcccccx cx m	CATTTTCCCC
	GGAAGTTGCT	CGCGAGGCAC	CGCGCGCACC	ACCCCCTCTA	GTAAAAGGGG
			000000000000000000000000000000000000000	ACCOCCIGIA	GINAMAGGGG
24151	GAACGCCTGC	TTAAAACCCT	GCAACAGGGT	CTGCCAGACT	TCACCAGTCA
	CTTGCGGÃCG	AATTTTGGGA	CGTTGTCCCA	GACGGTCTGA	AGTGGTCAGT
24201	3 3 CC 3 DCDDC	0.0			
24201	TTCCTDCDDC	CAGAACTTTA	GGAACTTTAT	CCTAGAGCGC	TCAGGAATCT AGTCCTTAGA
		GICII GELMI	CCIIGAAAIA	GGATCTCGCG	AGICCTIAGA
24251	TGCCCGCCAC	CTGCTGTGCA	CTTCCTAGCG	ACTTTGTGCC	CATTAAGTAC
	ACGGGCGGTG	GACGACACGT	GAAGGATCGC	TGAAACACGG	GTAATTCATG
24201	00000				
24301	CCCCCTTACCC	CACCCCCCCC	TTGGGGCCAC	TGCTACCTTC	TGCAGCTAGC ACGTCGATCG
	GCGCTTACGG	GAGGCGGCGA	AACCCCGGTG	ACGATGGAAG	ACGTCGATCG
24351	CAACTACCTT	GCCTACCACT	CTGACATAAT	GGAAGACGTG	AGCGGTGACG
	GTTGATGGAA	CGGATGGTGA	GACTGTATTA	CCTTCTGCAC	TCGCCACTGC
24401	GTCTACTGGA	GTGTCACTGT	CGCTGCAACC	TATGCACCCC	GCACCGCTCC
	CAGATGACCT	CACAGTGACA	GCGACGTTGG	ATACGTGGGG	CGTGGCGAGG
24451	CTGGTTTGCA	ATTCGCAGCT	GCTTAACGAA	እርጥ ር እ አ ስጥን አ	TO COMPA COMPA
	GACCAAACGT	TAAGCGTCGA	CGAATTGCTT	TCAGTTTAAT	AGCCATGGAA
24501	TGAGCTGCAG	GGTCCCTCGC	CTGACGAAAA	GTCCGCGGCT	CCGGGGTTGA
	ACTCGACGTC	CCAGGGAGCG	GACTGCTTTT	CAGGCGCCGA	GGCCCCAACT
24551	AACTCACTCC	GGGGCTGTGT	A CCIVCCCOMM	አ ርርጥጥር ርጉ እ	ADDDOM ACC
	TTGAGTGAGG	CCCCGACACC	TGCAGCCTT	TCCAACCCTT	TAAACATACCT

Figure 27Z

24521	GAGGACTACC	A CONCO	ር አ መመ አ ር ረር መጥር	TACCAACACC	AATCCCCCCC
24601	CTCCTGATGG	TGCGGGTGCT	CTAATCCAAG	ATGCTTCTGG	TTAGGGCGGG
	0.000				•
24651			CCTGCGTCAT		
	CGGATTACGC	CTCGAATGGC	GGACGCAGTA	ATGGGTCCCG	GTGTAAGAAC
24701		3.CCC3.0C3.3.C	AAAGCCCGCC	አአርአርምምምርም	CCTACCAAAC
24701			TTTCGGGCGG		
	CGG11121CG1		111000000		
24751			CCCCCAGTCC		
	CCTGCCCCCC	AAATGAACCT	GGGGGTCAGG	CCGCTCCTCG	AGTIGGGTTA
04001		0000) 0000	ATCAGCAGCA	000000000	CBBCCBBCCC
24801			TAGTCGTCGT		
	6666666666	GGCGTCGGGA	INGICOICGI	CGGCGCCCGG	arcarioo
24851	AGGATGGCAC	CCAAAAAGAA	GCTGCAGCTG	CCGCCGCCAC	CCACGGACGA
	TCCTACCGTG	GGTTTTTCTT	CGACGTCGAC	GCCGCCGTG	GGTGCCTGCT
					0,00,00,00
24901			AGGCAGAGGA TCCGTCTCCT		
	CCTCCTTATG	ACCCIGICAG	iccorcicci	CCAAAACCIG	CICCICCICC
24951	AGGACATGAT	GGAAGACTGG	GAGAGCCTAG	ACGAGGAAGC	TTCCGAGGTC
	TCCTGTACTA	CCTTCTGACC	CTCTCGGATC	TGCTCCTTCG	AAGGCTCCAG
		•			
25001			ACCGTCACCC		
	CTTCTCCACA	GTCTGCTTTG	TGGCAGTGGG	AGCCAGCGTA	AGGGGAGCGG
25051	GGCGCCCCAG	AAATCGGCAA	CCGGTTCCAG	CATGGCTACA	ACCTCCGCTC
			GGCCAAGGTC		
•					
25101			CCCGTTCGCC		
	GAGTCCGCGG	CGGCCGTGAC	GGGCAAGCGG	CIGGGTIGGC	ATCTACCCTG
25151	ACCACTGGAA	CCAGGGCCGG	TAAGTCCAAG	CAGCCGCCGC	CGTTAGCCCA
23131			ATTCAGGTTC		
25201			GCTACCGCTC		
	TCTCGTTGTT	GTCGCGGTTC	CGATGGCGAG	TACCGCGCCC	GIGITCIIGC
25251	ርር አጥ አርጥጥርር	ТТССТТССАА	GACTGTGGGG	GCAACATCTC	CTTCGCCCGC
23271	GGTATCAACG	AACGAACGTT	CTGACACCCC	CGTTGTAGAG	GAAGCGGGCG
25301			CGGCGTGGCC		
	GCGAAAGAAG	AGATGGTAGT	GCCGCACCGG	AAGGGGGCAT	TGTAGGACGT
25251	mma cma c c cm	רא תיריתיתיא רא	CCCCATACTC	CACCECCEC	AGCGGCAGCA
25351	AATGATGGCA	GTAGAGATGT	CGGGTATGAC	GTGGCCGCCG	TCGCCGTCGT
25401	ACAGCAGCGG	CCACACAGAA	GCAAAGGCGA	CCGGATAGCA	AGACTCTGAC
	TGTCGTCGCC	GGTGTGTCTT	CGTTTCCGCT	GGCCTATCGT	TCTGAGACTG
25.454	*******	*********	00000000000000	おたいなたにおたこ か	GGAGCGCTGC
25451	AAAGCCCAAG	TTTACCHCAG	GCCGCCGTCG	TCGTCCTCCT	CCTCGCGACG
					•
25501	GTCTGGCGCC	CAACGAACCC	GTATCGACCC	GCGAGCTTAG	AAACAGGATT
	CAGACCGCGG	GTTGCTTGGG	CATAGCTGGG	CGCTCGAATC	TTTGTCCTAA

Figure 27. AA

25551	TTTCCCACTC AAAGGGTGAG	TO TGCTAT ACATACGATA	ATTTCAACAG TAAAGTTGTC	AGCAGGGGCC TCGTCCCCGG	AAGAACA A TTCTTGTTCT
25601	GCTGAAAATA CGACTTTTAT	AAAAACAGGT TTTTTGTCCA	CTCTGCGATC GAGACGCTAG	CCTCACCCGC GGAGTGGGCG	AGCTGCCTGT TCGACGGACA
25651		CGAAGATCAG GCTTCTAGTC			
25701		AATACTGCGC TTATGACGCG			
25751		TAAGCGCGAA ATTCGCGCTT			
25801		TGTTGTCAGC ACAAÇAGTCG			
25851		GTTACCAGCC CAATGGTCGG			
25901		ACCCGAATAA TGGGCTTATT			
25951		CGGAATACGC GCCTTATGCG			
26001		CCACCACACC GGTGGTGTGG			
26051		GTGTACCAGG CACATGGTCC			
26101		CCAGGCCGAA GGTCCGGCTT			
26151		TTCGTCACAG AAGCAGTGTC			
26201		AGAGGGCGAG TCTCCCGCTC			
26251		TCTCCGTCCG AGAGGCAGGC			
26301	CGCTCTTCAT GCGAGAAGTA				AGACCTCGTC TCTGGAGCAG
26351	CTCTGAGCCG GAGACTCGGC				ATTGAGGAGT TAACTCCTCA
26401	TTGTGCCATC AACACGGTAG				CGGCCACTAT GCCGGTGATA
26451	CCGGATCAAT GGCCTAGTTA				

Figure 27 AB

26501	CTACGACTGA GATGCTGACT	A TAAGTG TACAATTCAC	GAGAGGCAGA CTCTCCGTCT	GCAACTGCGC CGTTGACGCG	CTGAAA C GACTTTGTGG
26551		TOGCOGCCAC AGCGGCGGTG			
26601	TGCTACTTTG	AATTGCCCGA	GGATCATATC	GAGGGCCCGG	CGCACGGCGT
	ACGATGAAAC	TTAACGGGCT	CCTAGTATAG	CTCCCGGGCC	GCGTGCCGCA
26651	CCGGCTTACC	GCCCAGGGAG	AGCTTGCCCG	TAGCCTGATT	CGGGAGTTTA
	GGCCGAATGG	CGGGTCCCTC	TCGAACGGGC	ATCGGACTAA	GCCCTCAAAT
26701	CCCAGCGCCC	CCTGCTAGTT	GAGCGGGACA	GGGGACCCTG	TGTTCTCACT
	GGGTCGCGGG	GGACGATCAA	CTCGCCCTGT	CCCCTGGGAC	ACAAGAGTGA
26751	GTGATTTGCA	ACTGTCCTAA	CCCTGGATTA	CATCAAGATC	TTTGTTGCCA
	CACTAAACGT	TGACAGGATT	GGGACCTAAT	GTAGTTCTAG	AAACAACGGT
26801	TCTCTGTGCT AGAGACACGA	GAGTATAATA CTCATATTAT	AATACAGAAA TTATGTCTTT	ATATAAATTA TATATTTAA	CTGGGGCTCC GACCCCGAGG
26851	TATCGCCATC	CTGTAAACGC	CACCGTCTTC	ACCCGCCCAA	GCAAACCAAG
	ATAGCGGTAG	GACATTTGCG	GTGGCAGAAG	TGGGCGGGTT	CGTTTGGTTC
26901	GCGAACCTTA	CCTGGTACTT	TTAACATCTC	TCCCTCTGTG	ATTTACAACA
	CGCTTGGAAT	GGACCATGAA	AATTGTAGAG	AGGGAGACAC	TAAATGTTGT
26951	GTTTCAACCC	AGACGGAGTG	AGTCTACGAG	AGAACCTCTC	CGAGCTCAGC
	CAAAGTTGGG	TCTGCCTCAC	TCAGATGCTC	TCTTGGAGAG	GCTCGAGTCG
27001	TACTCCATCA	GAAAAAACAC	CACCCTCCTT	ACCTGCCGGG	AACGTACGAG
	ATGAGGTAGT	CTTTTTTGTG	GTGGGAGGAA	TGGACGGCCC	TTGCATGCTC
27051	TGCGTCACCG	GCCGCTGCAC	CACACCTACC	GCCTGACCGT	AAACCAGACT
	ACGCAGTGGC	CGGCGACGTG	GTGTGGATGG	CGGACTGGCA	TTTGGTCTGA
27101	TTTTCCGGAC	AGACCTCAAT	AACTCTGTTT	ACCAGAACAG	GAGGTGAGCT
	AAAAGGCCTG	TCTGGAGTTA	TTGAGACAAA	TGGTCTTGTC	CTCCACTCGA
27151					GTGGGGTTTA CACCCCAAAT
27201	TGAACAATTC	AAGCAACTCT	ACGGGCTATT	CTAATTCAGG	TTTCTCTAGA
	ACTTGTTAAG	TTCGTTGAGA	TGCCCGATAA	GATTAAGTCC	AAAGAGATCT
27251	ATCGGGGTTG	GGGTTATTCT	CTGTCTTGTG	ATTCTCTTTA	TTCTTATACT
	TAGCCCCAAC	CCCAATAAGA	GACAGAACAC	TAAGAGAAAT	AAGAATATGA
27301	AACGCTTCTC	TGCCTAAGGC	TCGCCGCCTG	CTGTGTGCAC	ATTTGCATTT
	TTGCGAAGAG	ACGGATTCCG	AGCGGCGGAC	GACACACGTG	TAAACGTAAA
27351	ATTGTCAGCT	TTTTAAACGC	TGGGGTCGCC	ACCCAAGATG	ATTAGGTACA
	TAACAGTCGA	AAAATTTGCG	ACCCCAGCGG	TGGGTTCTAC	TAATCCATGT
27401	TAATCCTAGG ATTAGGATCC	TTTACTCACC AAATGAGTGG	CTTGCGTCAG GAACGCAGTC	CCCACGGTAC	CACCCAAAAG GTGGGTTTTC

Ligure 27AC

27451	GTGGATTŢTA	ACCOCCAGO	CTGTAATGTT GACATTACAA	ACATTCGCAG	CTGAAG A
	CACCIAAAAI	1CCTCGG1CG	GACATTACAA	TGTAAGCGTC	GACTTCGATT
27501	TGAGTGCACC ACTCACGTGG	ACTCTTATAA TGAGAATATT	AATGCACCAC TTACGTGGTG	AGAACATGAA TCTTGTACTT	AAGCTGCTTA TTCGACGAAT
27551	TTCGCCACAA	AAACAAAATT	GGCAAGTATG	CTGTTTATGC	TATTTGGCAG
	AAGCGGTGTT	TTTGTTTAA	CCGTTCATAC	GACAAAȚACG	ATAAACCGTC
27601			TAATGTTACA ATTACAATGT		
27651			TTCCATTTTA		
	ATTTTGAAAA	TACATATGAA	AAGGTAAAAT	ACTTTACACG	CTGTAATGGT
27701	TGTACATGAG ACATGTACTC	CAAACAGTAT GTTTGTCATA	AAGTTGTGGC TTCAACACCG	CCCCACAAAA	TTGTGTGGAA
27751	AACACTGGCA TTGTGACCGT	CTTTCTGCTG	CACTGCTATG GTGACGATAC	CTAATTACAG	TGCTCGCTTT
		•			
27801	GGTCTGTACC	CTACTCTATA	TTAAATACAA	AAGCAGACGC	AGCTTTATTG
	CCAGACATGG	GATGAGATAT	AATTTATGTT	TTCGTCTGCG	TCGAAATAAC
27851	AGGAAAAGAA	AATGCCTTAA	TTTACTAAGT	TACAAAGCTA	ATGTCACCAC
	TCCTTTTCTT	TTACGGAATT	AAATGATTCA	ATGTTTCGAT	TACAGTGGTG
27901	TAACTGCTTT	ACTCGCTGCT	TGCAAAACAA	ATTCAAAAAG	TTAGCATTAT
			ACGTTTTGTT		
27951	AATTAGA <u>A</u> TA	_GGATTTAAAC	CCCCCGGTCA	TTTCCTGCTC	AATACCATTC
	TTÄATCTTAT	CCTAAATTTG	GGGGGCCAGT	AAAGGACGAG	TTATGGTAAG
28001			GTGGGATATG		
	GGGACTTGTT	AACTGAGATA	CACCCTATAC	GAGGTCGCGA	TGTTGGAACT
28051			AGCATCTGAC		
	TCAGTCCGAA	GGACCTACAG	TCGTAGACTG	AAACCGGTCG	TGGACAGGGC
28101	CGGATTTGTT	CCAGTCCAAC	TACAGCGACC	CACCCTAACA	GAGATGACCA
	GCCTAAACAA	GGTCAGGTTG	ATGTCGCTGG	GTGGGATTGT	CTCTACTGGT
28151	ACACAACCAA	CGCGGCCGCC	GCTACCGGAC	ТТАСАТСТАС	CACAAATACA
			CGATGGCCTG		
28201	CCCCAAGTTT	CTGCCTTTGT	CAATAACTGG	GATAACTTGG	GCATGTGGTG
	GGGGTTCAAA	GACGGAAACA	GTTATTGACC	CTATTGAACC	CGTACACCAC
28251	GTTCTCCATA	GCGCTTATGT	TTGTATGCCT	TATTATTATG	TGGCTCATCT
	CAAGAGGTAT	CGCGAATACA	AACATACGGA	ATAATAATAC	ACCGAGTAGA
28301	GCTGCCTAAA				
	CGACGGATTT	CCCCTTTCCC	CGGGCTGGTG	GGTAGATATC	AGGGTAGTAA
28351	GTGCTACACC	CAAACAATGA	TGGAATCCAT	AGATTGGACG	GACTGAAACA
	CACGATGTGG				

Figure 27AD

28401	CATGTTCTTT	TTACAG	TATGATTAAA	TGAGACATGÃ	TTCCTC
20302	GTACAAGAAA .	AGAGAATGTC	ATACTAATTT	ACTCTGTACT	AAGGAGCTCA
28451	TTTTATATTA	CTGACCCTTG	TTGCGCTTTT	TTGTGCGTGC	TCCACATTGG
	AAAATATAAT	GACTGGGAAC	AACGCGAAAA	AACACGCACG	AGGTGTAACC
28501	CTGCGGTTTC	TCACATCGAA	GTAGACTGCA	TTCCAGCCTT	CACAGTCTAT
	GACGCCAAAG	AGTGTAGCTT	CATCTGACGT	AAGGTCGGAA	GTGTCAGATA
28551	TTGCTTTACG	GATTTGTCAC	CCTCACGCTC	ATCTGCAGCC	TCATCACTGT
	AACGAAATGC	CTAAACAGTG	GGAGTGCGAG	TAGACGTCGG	AGTAGTGACA
28601	GGTCATCGCC	TTTATCCAGT	GCATTGACTG	GGTCTGTGTG	CGCTTTGCAT
	CCAGTAGCGG	AAATAGGTCA	CGTAACTGAC	CCAGACACAC	GCGAAACGTA
28651	ATCTCAGACA	CCATCCCCAG	TACAGGGACA	GGACTATAGC	TGAGCTTCTT
	TAGAGTCTGT	GGTAGGGGTC	ATGTCCCTGT	CCTGATATCG	ACTUGAAGAA
					as mins mmmaa
28701	AGAATTCTTT	AATTATGAAA	TTTACTGTGA	CTTTTCTGCT	GATTATTTGC
	TCTTAAGAAA	TTAATACTTT	AAATGACACT	GAAAAGACGA	CTAATAAACG
				3 3 CCCCCC 3 3 3	CACAMAMAMC
28751	ACCCTATCTG	CGTTTTGTTC	CCCGACCTCC	AAGCCTCAAA	CUCUNTATALC
	TGGGATAGAC	GCAAAACAAG	GGGCTGGAGG	TTCGGAGTTT	CIGIAIAIAG
			CC > MARINCC	AAGTTGCTAC	מממממתמת
28801	ATGCAGATTC	ACTCGTATAT	GGAATATTCC	TTCAACGATG	Wat 7 Granden Anna Lange
	TACGTCTAAG	TGAGCATATA	CCTTATAAGG	IICANCGAIG	· ·
		0033000000	መመን መንጥር ር ል ል	ጥር አጥር ጥር ጥር ጥ	TATGGTGTTC
28851	GCGATCTTTC	CCMAGCCIGG	11M1M1GCM1	AGTAGAGACA	ATACCACAAG
	CGCTAGAAAG	GCTTCGGACC	AMIMIACOII	,10111011011	
20003	mac y cm y cc y	ጥር ጥጥ አር ቦር ቦር ጥ	ΤΑΨΑΨΑΨΟΩΑ	CCCTACCTTG	ACATTGGCTG
28901	1GCAGTACCA	ACANTOGGA	TCCATATATA	GGGATGGAAC	TGTAACCGAC
	ACGICAIGGI	AGAATCGGGA	100111111		
28951	ごかかごごごみを作る	CATCCCATGA	ACCACCCAAC	TTTCCCCGCG	CCCGCTATGC
70371	CHACCCCATA	СТАСССТАСТ	TGGTGGGTTG	AAAGGGGCGC	GGGCGATACG
	CIIGCGIIA	C11100011101			
29001	TTCCACTGCA	ACAAGTTGTT	GCCGGCGGCT	TTGTCCCAGC	CAATCAGCCT
23001	AAGGTGACGT	TGTTCAACAA	CGGCCGCCGA	AACAGGGTCG	GTTAGTCGGA
29051	CGCCCACCTT	CTCCCACCCC	CACTGAAATC	AGCTACTTTA	ATCTAACAGG
	GCGGGTGGAA	GAGGGTGGGG	GTGACTTTAG	TCGATGAAAI	TAGATTGTCC
	•				
29101	AGGAGATGAC	TGACACCCTA	GATCTAGAAA	TGGACGGAAT	TATTACAGAG
	TCCTCTACTG	ACTGTGGGAT	CTAGATCTTT	ACCTGCCTTA	ATAATGTCTC
29151	CAGCGCCTGC	TAGAAAGACG	CAGGGCAGCG	GCCGAGCAAC	AGCGCATGAA
	GTCGCGGACG	ATCTTTCTGC	GTCCCGTCGC	CGGCTCGTTG	TCGCGTACTT
29201	TCAAGAGCTC	CAAGACATGO	TTAACTTGC	CCAGTGCAA	AGGGGTATCT
	AGTTCTCGAG	GTTCTGTACC	AATTGAACGT	CGTCACGTT	TCCCCATAGA
29251	TTTGTCTCGT	AAAGCAGGCC	AAAGTCACCT	r ACGACAGTA	TACCACCGGA
	AAACAGAGCA	TTTCGTCCG	TTTCAGTGG/	A TGCTGTCAT	ATGGTGGCCT
29301	CACCGCCTTA	GCTACAAGTT	CCCAACCAAC	G CGTCAGAAA	TACTOSTOST
	GTGGCGGAAT	CGATGTTCA	A CGGTTGGTT(C GCAGTCTTT	ACCACCAGTA

Figure 27 AE

29351	GGTGGGAGAA	ACCATTA	CCATAACTCA	GCACTCGGTA	GAAACC
		TTCGGGTAAT			
29401	GCTGCATTCA	CTCACCTTGT	CAAGGACCTG	AGGATCTCTG	CACCCTTATT
	CGACGTAAGT	GAGTGGAACA	GTTCCTGGAC	TCCTAGAGAC	GTGGGAATAA
29451		GCGGTCTCAA			
	TTCTGGGACA	CGCCAGAGTT	TCTAGAATAA	GGGAAATTGA	TTATTTTTTT
29501	AATAATAAAG	CATCACTTAC	TTAAAATCAG	TTAGCAAATT	TCTGTCCAGT
	TTATTATTTC	GTAGTGAATG	AATTTTAGTC	AATCGTTTAA	AGACAGGTCA
29551	TTATTCAGCA	GCACCTCCTT	GCCCTCCTCC	CAGCTCTGGT	ATTGCAGCTT
	AATAAGTCGT	CGTGGAGGAA	CGGGAGGAGG	GTCGAGACCA	TAACGTCGAA
29601	CCTCCTGGCT	GCAAACTTTC	TCCACAATCT	AAATGGAATG	TCAGTTTCCT
	GGAGGACCGA	CGTTTGAAAG	AGGTGTTAGA	TTTACCTTAC	AGTCAAAGGA
29651	CCTGTTCCTG	TCCATCCGCA	CCCACTATCT	TCATGTTGTT	GCAGATGAAG
		AGGTAGGCGT			
29701	CGCGCAAGAC	CGTCTGAAGA	TACCTTCAAC	CCCGTGTATC	CATATGACAC
	GCGCGTTCTG	GCAGACTTCT	ATGGAAGTTG	GGGCACATAG	GTATACTGTG
29751	GGAAACCGGT	CCTCCAACTG	TGCCTTTTCT	TACTCCTCCC	TTTGTATCCC
	CCTTTGGCCA	GGAGGTTGAC	ACGGAAAAGA	ATGAGGAGGG	AAACATAGGG
29801	CCAATGGGTT	TCAAGAGAGT	CCCCTGGGG	TACTCTCTTT	GCGCCTATCC
	GGTTACCCAA	AGTTCTCTCA	GGGGGACCCC	ATGAGAGAAA	CGCGGATAGG
29851	GAACCTCTAG	TTACCTCCAA	TGGCATGCTT	GCGCTCAAAA	TGGGCAACGG
	CTTGGAGATC	AATGGAGGTT	ACCGTACGAA	CGCGAGTTTT	ACCCGTTGCC
29901		GACGAGGCCG			
		CTGCTCCGGC		•	
29951	TGAGCCCACC	TCTCAAAAAA	ACCAAGTCAA	ACATAAACCT	GGAAATATCT
	ACTCGGGTGG	AGAGTTTTTT	TGGTTCAGTT	TGTATTTGGA	CCTTTATAGA
30001		CAGTTACCTC			
		GTCAATGGAG			
30051	TCTAATGGTC	GCGGGCAACA	CACTCACCAT	GCAATCACAG	GCCCCGCTAA
	AGATTACCAG	CGCCCGTTGT	GTGAGTGGTA	CGTTAGTGTC	CGGGGCGATT
30101	CCGTGCACGA	CTCCAAACTT	AGCATTGCCA	CCCAAGGACC	CCTCACAGTG
	GGCACGTGCT	GAGGTTTGAA	TCGTAACGGT	GGGTTCCTGG	GGAGTGTCAC
30151	TCAGAAGGAA				
	AGTCTTCCTT				
30201	TAGCAGTACC				
	ATCGTCATGG				
30251	GTAGCTTGGG	CATTGACTTG	AAAGAGCCCA	TTTATACACA	AAATGGAAAA
		GTAACTGAAC			

Figure 27 AF

30301		TCGCCCCG			
30351	TTTGACCGTA AAACTGGCAT	GCAACTGGTC CGTTGACCAG			
30401		TACTGGAGCC ATGACCTCGG			
30451	CTTAATGTAG	CAGGAGGACT	AAGGATTGAT	TCTCAAAACA	GACGCCTTAT
20501		GTCCTCCTGA AGTTATCCGT			
30501	TGAACTACAA				
30551		CCCTCTTTTT			
30601		GCCTTTACTT CGGAAATGAA			
30651		CTAAGCACTG GATTCGTGAC			
30701	TAGCCATTAA	TGCAGGAGAT	GGGCTTGAAT	TTGGTTCACC	TAATGCACCA
		ACGTCCTCTA CCCTCAAAAC			•
30751		GGGAGTTTTG			
30801		ATGGTTCCTA TACCAAGGAT			
30851		TACAGTAGGA ATGTCATCCT			
30901		CTCCATCTCC GAGGTAGAGG			AGAAAGATGC TCTTTCTACG
30951					CTTGCTACAG GAACGATGTC
31001	TTTCAGTTTT	GGCTGTTAAA	GGCAGTTTGG	CTCCAATATC	TGGAACAGTT
21.054					ACCTTGTCAA TGCTACTAAA
	GTTTCACGAG	TAGAATAATA	TTCTAAACTG	CTTTTACCTC	ACGATGATTT
31101	CAATTCCTTC GTTAAGGAAG	CTGGACCCAG GACCTGGGTC	AATATTGGAA TTATAACCTT	CTTTAGAAAT GAAATCTTTA	GGAGATCTTA CCTCTAGAAT
31151	CTGAAGGCAC GACTTCCGTG	AGCCTATACA TCGGATATGT	AACGCTGTTG TTGCGACAAC	GATTTATGCC CTAAATACGG	TAACCTATCA ATTGGATAGT
31201	GCTTATCCAA CGAATAGGTT	AATCTCACGG	TAAAACTGCC ATTTTGACGG	AAAAGTAACA TTTTCATTGT	TTGTCAGTCA AACAGTCAGT

Figure 27 AG

31251	AGTTTACTTA	AREGGAGACA	AAACTAAACC	TGTAACACTA	ACCATTAGAC
	TCAAATGAAT	TTGCCTCTGT	TTTGATTTGG	ACATTGTGAT	TGGTAATGTG
•					
21201	TAAACGGTAC	ACACCAAACA	CCACACACAA	CMCCX X CMCC	እ ጥ እ ርጥር m እ ጠር
31301					
	ATTTGCCATG	TGTCCTTTGT	CCTCTGTGTT	GAGGTTCACG	TATGAGATAC
31351	TCATTTTCAT	GGGACTGGTC	TGGCCACAAC	TACATTAATG	AAATATTTGC
	AGTAAAAGTA	CCCTGACCAG	ACCGGTGTTG	ATGTAATTAC	TTTATAAACG
31401	CACATCCTCT	TACACTTTTT	САТАСАТТСС	ССАВСВАТАВ	ልሮልልጥሮርጥጥጥ
	•	ATGTGAAAAA			
	GIGINGGNGN	MIGIGINAM	GIAIGIAACG	GGIICIIAII	ICIINGCAAA
	424221 B	~~~			
31451		TCAACGTGTT			
	CACAATACAA	AGTTGÇACAA	ATAAAAAGTT	AACGTCTTTT	AAAGTTCAGT
	•				
31501	TTTTTCATTC	AGTAGTATAG	CCCCACCACC	ACATAGCTTA	TACAGATCAC
	AAAAAGTAAG	TCATCATATC	GGGGTGGTGG	TGTATCGAAT	ATGTCTAGTG
31551	ССТАССТТА	TCAAACTCAC	ACAACCCTAC	תוא שישיר א א ריכיתי	CCCXCCTCCC
31331					
	GCATGGAATT	AGTTTGAGTG	TCTTGGGATC	ATAAGTTGGA	CGGTGGAGGG
31601	TCCCAACACA	CAGAGTACAC	AGTCCTTTCT	CCCCGGCTGG	CCTTAAAAAG
	AGGGTTGTGT	GTCTCATGTG	TCAGGAAAGA	GGGGCCGACC	GGAATTTTTC
31651	CATCATATCA	TGGGTAACAG	ACATATTCTT	AGGTGTTATA	TTCCACACGG
		ACCCATTGTC			
	GIAGIAIAGI	ACCCATIGIC	IGINIANGAN	ICCACAAIAI	Magraracc
24.204					
31701		AGCCAAACGC			
	AAAGGACAGC	TCGGTTTGCG	AGTAGTCACT	ATAATTATTT	GAGGGGCCCG
31751	AGCTCACTTA	AGTTCATGTC	GCTGTCCAGC	TGCTGAGCCA	CAGGCTGCTG
	TCGAGTGAAT	TCAAGTACAG	CGACAGGTCG	ACGACTCGGT	GTCCGACGAC
31801	ጥርር እ አርጥጥርር	GGTTGCTTAA	CCCCCCCC*	ACCACAACTY	CACGCCTACA
51001	•	CCAACGAATT			
	MOGIIGAMCG	CCMACGMATT	GCCCGC1	ICCICITCAG	GIGCGGAIGI
31851		GTCATAATCG			
	ACCCCCATCT	CAGTATTAGC	ACGTAGTCCT	ATCCCGCCAC	CACGACGTCG
31901	AGCGCGCGAA	TAAACTGCTG	CCGCCGCCGC	TCCGTCCTGC	AGGAATACAA
		ATTTGACGAC			
	1000000011	Al II Conconc	0000000000	1100011001100	
21051	01 m0001 0m0	000000000000000000000000000000000000000	001 001 0000	03.00000000	
31951		GTCTCCTCAG			
	GTACCGTCAC	CAGAGGAGTC	GCTACTAAGC	GTGGCGGGCG	TCGTATTCCG
32001	GCCTTGTCCT	CCGGGCACAG	CAGCGCACCC	TGATCTCACT	TAAATCAGCA
	CGGAACAGGA	GGCCCGTGTC	GTCGCGTGGG	ACTAGAGTGA	ATTTAGTCGT
32051	CAGTAACTGC	ACCACACCAC	ሮልሮልልጥልጥጥር	ምምር ል ል ል ጥር ር	СУСУСТСТУУ
22021					GTGTCACGTT
	GICATIGACG	100101010	GIGITATAAC	AMGTTTTAGG	GIGICACGIT
32101	GGCGCTGTAT				
	CCGCGACATA	GGTTTCGAGT	ACCGCCCCTG	GTGTCTTGGG	TGCACCGGTA
32151	CATACCACAA	GCGCAGGTAG	ATTAAGTGGC	GACCCCTCAT	AAACACGCTG
					TTTGTGCGAC

Figure 27 AH

32201		CTCTTT			
	CTGTATTTGT	AATGGAGAAA	ACCGTACAAC	ATTAAGTGGT	GGAGGGCCAT
32251	CCATATAAAC	CTCTGATTAA	ACATRICCRIC	ATCCACCACC	ATCCTAAACC
32231		GAGACTAATT			
•				•	
32301	AGCTGGCCAA	AACCTGCCCG	CCGGCTATAC	ACTGCAGGGA	ACCGGGACTG
	TCGACCGGTT	TTGGACGGGC	GGCCGATATG	TGACGTCCCT	TGGCCCTGAC
32351	CARCARTERO	AGTGGAGAGC	CCAGGACTCG	TAACCATGGA	TCATCATGCT
32331		TCACCTCTCG			
	*****	TCAATGTTGG	0.00.0.0	002 02 00000	>m><>>CDTCC
32401					
	GCAGTACTAT	AGTTACAACC	GTGTTGTGTC	CGIGIGCACG	TATGTGAAGG
32451	TCAGGATTAC	AAGCTCCTCC	CGCGTTAGAA	CCATATCCCA	GGGAACAACC
		TTCGAGGAGG			
20501	C> mmccmc> >	TCAGCGTAAA	mcccacacacac	CACCCAACAC	בתיכנים בניים
32501					
		AGTCGCATTT		•	
32551	ACTCACGTTG	TGCATTGTCA	AAGTGTTACA	TTCGGGCAGC	AGCGGATGAT
	TGAGTGCAAC	ACGTAACAGT	TTCACAATGT	AAGCCCGTCG	TCGCCTACTA
2000	00000000000	GGTAGCGCGG	CMMMCMCMCM	CAAAACCACC	TACACCATIC
32601					
		CCATCGCGCC			
32651	CTACTGTACG	GAGTGCGCCG	AGACAACCGA	GATCGTGTTG	GTCGTAGTGT
	GATGACATGC	CTCACGCGGC	TCTGTTGGCT	CTAGCACAAC	CAGCATCACA
32701	CATCCCA 3 3 T	GC / MCCCCGG	አ ርርጥ <u>አርጥር</u> አጥ	<u>አጥጥጥና ሮጥር እ</u> እ	GCAAAACCAG
32/01	CWIGCCWW	CCTTGCGGCC	DCC3DC3CD3	TO A A C C A C TUT	COMPANICATO
32751	GTGCGGGCGT	GACAAACAGA	TCTGCGTCTC	CGGTCTCGCC	GCTTAGATCG
	CACGCCCGCA	CTGTTTGTCT	AGACGCAGAG	GCCAGAGCGG	CGAATCTAGC
32801	СФСФСФСФАС	TAGTTGTAGT	ልጥልጥርር እርጥር	ጥርጥር እ A A GCA	TCCAGGCGCC
32001					AGGTCCGCGG
32851					TGCCCTGATA
	GGGACCGAAG	CCCAAGATAC	ATTTGAGGAA	GTACGCGGCG	ACGGGACTAT
32901	אראשירטרט	ССССРСРЭТР	AGCCACACCC	AGCCAACCTA	CACATTCGTT
32901	TGTAGGTGGT	GGCGTCTTAT	TCGGTGTGGG	TCGGTTGGAT	GTGTAAGCAA
			a. caaaa a	. ccmcc c.	>00>m0mmm
32951	CTGCGAGTCA	CACACGGGAG	GAGCGGGAAG	AGCTGGAAGA	ACCATGTTTT
	GACGCTCAGT	GTGTGCCCTC	CTCGCCCTTC	TCGACCTTCT	TGGTACAAAA
33001	TALTALALAL	CCAAAAGATT	ATCCAAAACC	TCAAAATGAA	GATCTATTAA
	AAAAAAATAA	GGTTTTCTAA	TAGGTTTTGG	AGTTTTACTT	CTAGATAATT
		managemenee	The Colonia Company	እ <i>እ እ ርተ</i> መንጠአቦ እ	CCCABAGAAC
33051	GTGAACGCGC	TUCULTUCGG	100001001C	AMACICIACA	GCCAAAGAAC
					CGGTTTCTTG
33101	AGATAATGGC	ATTTGTAAGA	TGTTGCACAA	TGGCTTCCAA	AAGGCAAACG
	TCTATTACCG	TAAACATTCT	ACAACGTGTT	ACCGAAGGTT	TTCCGTTTGC

Figure 27 AI

33151	G CACCTG		
33201	ATTCCAGCAC TAAGGTCGTG	 	
33251	 CAATATATCT GTTATATAGA	 	
33301	 TCTGCTCCAG AGACGAGGTC	 	
33351	 GCAAAAATTC CGTTTTTAAG	 	
33401	 TTAACAAAAA AATTGTTTTT	 	
33451	 ATAATCGTGC TATTAGCACG	 	
33501	CCATGACAAA GGTACTGTTT		
33551	 CTAACCAGCG GATTGGTCGC	 	
33601	ATGCAAGGTG TACGTTCCAC		
33651	GCACATCGTA CGTGTAGCAT		
33701	 ACCACAGAAA TGGTGTCTTT	 	
33751	 CATAAACACA GTATTTGTGT	 	
3380i	 TCTTACAACA AGAATGTTGT		-
33851	 TGCCGGCGTG ACGGCCGCAC		
33901			ATGTAAGACT TACATTCTGA
33951			AAAGCGACCG TTTCGCTGGC
34001			ACATTACAGC TGTAATGTCG
34051			ACATAAACAC TGTATTTGTG

Figure 27AJ

- 4	CTGAAAAACC I	an-dragger	CCCXXXXIIIXC	and commended a	
34101	GACTTTTTGG	G. ACGGAT	CCGTTTTATC	GTGGGAGGGC	GAGGTC T
34151	ACATACAGCG TGTATGTCGC	CTTCCACAGC GAAGGTGTCG	GGCAGCCATA CCGTCGGTAT	ACAGTCAGCC TGTCAGTCGG	TTACCAGTAA AATGGTCATT
	AAAAGAAAAC				
	TTTTCTTTTG	GATAATTTTT	TIGIGGIGAG	CIGIGCCGIG	GICGAGITAG
34251	AGTCACAGTG TCAGTGTCAC			AGCGAGTATA TCGCTCATAT	
34301	AAAAATGACG TTTTTACTGC			AAACACCCAG TTTGTGGGTC	
34351				AAACCCACAA TTTGGGTGTT	
34401	TCGTCACTTC AGCAGTGAAG	CGTTTTCCCA GCAAAAGGGT	CGTTACGTCA GCAATGCAGT	CTTCCCATTT GAAGGGTAAA	TAAGAAAACT ATTCTTTTGA
34451	ACAATTCCCA	ACACATACAA	GTTACTCCGC	ССТААААССТ	ACGTCACCCG
	TGTTAAGGGT	TGTGTATGTT	CAATGAGGCG	GGATTTTGGA	TGCAGTGGGC
34501	CCCCGTTCCC GGGGCAAGGG	ACGCCCCGCG TGCGGGGGGCGC	CCACGTCACA GGTGCAGTGT	AACTCCACCC TTGAGGTGGG	CCTCATTATC GGAGTAATAG
					PacI
34551	ATATTGGCTT TATAACCGAA	CAATCCAAAA GTTAGGTTTT	TAAGGTATAT ATTCCATATA	TATTGATGAT ATAACTACTA	GTTAATTAAG CAATTAATTC
34551	TATAACCGAA	GTTAGGTTTT	ATTCCATATA	TATTGATGAT ATAACTACTA CCTTCCCCAT GGAAGGGGTA	CAATTAATTC
	TATAACCGAA AATTCGGATC TTAAGCCTAG CTCGCTTCCG	GTTAGGTTTT TGCGACGCGA ACGCTGCGCT GCGGCATCGG	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA	CAATTAATTC TATGATTCTT ATACTAAGAA TGCTGTCCAG
34601	TATAACCGAA AATTCGGATC TTAAGCCTAG CTCGCTTCCG GAGCGAAGGC	GTTAGGTTTT TGCGACGCGA ACGCTGCGCT GCGGCATCGG CGCCGTAGCC	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGGCGC	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA AACGTCCGGT	CAATTAATTC TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC
34601	TATAACCGAA AATTCGGATC TTAAGCCTAG CTCGCTTCCG GAGCGAAGGC GCAGGTAGAT	GTTAGGTTTT TGCGACGCGA ACGCTGCGCT GCGGCATCGG CGCCGTAGCC	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGGCGC AGGGACAGCT	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA AACGTCCGGT TCAAGGCCAG	CAATTAATTC TATGATTCTT ATACTAAGAA TGCTGTCCAG
34601	TATAACCGAA AATTCGGATC TTAAGCCTAG CTCGCTTCCG GAGCGAAGGC GCAGGTAGAT CGTCCATCTA	TGCGACGCGA ACGCTGCGCT GCGGCATCGG CGCCGTAGCC GACGACCATC CTGCTGGTAG	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGGCGC AGGGACAGCT TCCCTGTCGA	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA AACGTCCGGT TCAAGGCCAG AGTTCCGGTC	CAATTAATTC TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC CAAAAGGCCA GTTTTCCGGT
34601 34651 34701 34751	TATAACCGAA AATTCGGATC TTAAGCCTAG CTCGCTTCCG GAGCGAAGGC GCAGGTAGAT CGTCCATCTA GGAACCGTAA CCTTGGCATT	TTAGGTTTT TGCGACGCGA ACGCTGCGCT GCGCCATCGG CGCCGTAGCC GACGACCATC CTGCTGGTAG AAAGGCCGCG TTTCCGGCGC	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGGCGC AGGGACAGCT TCCCTGTCGA TTGCTGGCGT AACGACCGCA	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA AACGTCCGGT TCAAGGCCAG AGTTCCGGTC TTTTCCATAG AAAAGGTATC	CAATTAATTC TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC CAAAAGGCCA GTTTTCCGGT GCTCCGCCCC CGAGGCGGGG
34601 34651 34701 34751 34801	TATAACCGAA AATTCGGATC TTAAGCCTAG CTCGCTTCCG GAGCGAAGGC GCAGGTAGAT CGTCCATCTA CCTTGGCATC CCTGACGAGC GGACTGCTCG GACAGGCTCG	GTTAGGTTTT TGCGACGCGA ACGCTGCGCT GCGCCATCGG CGCCGTAGCC GACGACCATC CTGCTGGTAG AAAGGCCGCG TTTCCGGCGC ATCACAAAAA TAGTGTTTTT	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGGCGC AGGGACAGCT TCCCTGTCGA TTGCTGGCGT AACGACCGCA TCGACGCTCA AGCTGCGAGT	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA AACGTCCGGT TCAAGGCCAG AGTTCCGGTC TTTTCCATAG AAAAGGTATC AGTCAGAGGT TCAGTCTCCA	CAATTAATTC TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC CAAAAGGCCA GTTTTCCGGT GCTCCGCCCC CGAGGCGGGG GGCGAAACCC CCGCTTTGGG
34651 34701 34751 34801 34851	TATAACCGAA AATTCGGATC TTAAGCCTAG CTCGCTTCCG GAGCGAAGGC GCAGGTAGAT CGTCCATCTA CCTTGGCATT CCTGACGAGC GGACTGCTCG GACAGGACTA CTGTCCTGAT CTGTCCTGAT	GTTAGGTTTT TGCGACGCGA ACGCTGCGCT GCGGCATCGG CGCCGTAGCC GACGACCATC CTGCTGGTAG AAAGGCCGCG ATCACAAAAA TAGTGTTTTT TAAAGATACC ATTTCTATGG	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGGCGC AGGGACAGCT TCCCTGTCGA TTGCTGGCGT AACGACGCTCA AGCTGCGAGT AGCTGCGAGT CCGCAAAGG	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA AACGTCCGGT TCAAGGCCAG AGTTCCGGTC TTTTCCATAG AAAAGGTATC AGTCAGAGGT TCAGTCTCCA CCCTGGAAGC GGGACCTTCG	CAATTAATTC TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC CAAAAGGCCA GTTTTCCGGT GCTCCGCCCC CGAGGCGGGG GCCAAACCC CCGCTTTGGG TCCCTCGTGC AGGGAGCACG
34651 34701 34751 34801 34851 34901	TATAACCGAA AATTCGGATC TTAAGCCTAG CTCGCTTCCG GAGCGAAGGC GCAGGTAGAT CGTCCATCTA CCTTGGCATCT CCTGACGAGC GGACTGCTCG GACAGGACTA CTGTCCTGAT CTGTCCTGAT CTGTCCTGAT CCTCTCGGAT	GTTAGGTTTT TGCGACGCGA ACGCTGCGCT GCGCCATCGG CGCCGTAGCC GACGACCATC CTGCTGGTAG AAAGGCCGCG ATCACAAAAA TAGTGTTTTT TAAAGATACC ATTTCTATGG TCCGACCCTG AGGCTGGCGCT	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGGCGC AGGGACAGCT TCCCTGTCGA TCCCTGTCGA ACGACCGCA ACGACCGCA ACGCCTTACCG CCGCAAAGG CCGCTTACCG CGCGAATGGC TTCTCATAGG	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA AACGTCCGGT TCAAGGCCAG AGTTCCGGTC TTTTCCATAG AAAAGGTATC AGTCAGAGGT TCAGTCTCCA CCCTGGAAGC CGGACCTTCG GATACCTGTC CTATGGACAG	TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC CAAAAGGCCA GTTTTCCGGT GCTCCGCCCC CGAGGCGGGG TCCCTCGTGC AGGGAGCACG CGCCTTTCTC GCGGAAACAG
34651 34701 34751 34801 34851 34901	TATAACCGAA AATTCGGATC TTAAGCCTAG CTCGCTTCCG GAGCGAAGGC GCAGGTAGAT CGTCCATCTA CCTTGGCATCT CCTGACGAGC GGACTGCTCG GACAGGACTA CTGTCCTGAT CTGTCCTGAT CTGTCCTGAT CCTCTCGGAT	GTTAGGTTTT TGCGACGCGA ACGCTGCGCT GCGCCATCGG CGCCGTAGCC GACGACCATC CTGCTGGTAG AAAGGCCGCG ATCACAAAAA TAGTGTTTTT TAAAGATACC ATTTCTATGG TCCGACCCTG AGGCTGGCGCT	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGGCGC AGGGACAGCT TCCCTGTCGA TCCCTGTCGA ACGACCGCA ACGACCGCA ACGCCTTACCG CCGCAAAGG CCGCTTACCG CGCGAATGGC TTCTCATAGG	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA AACGTCCGGT TCAAGGCCAG AGTTCCGGTC TTTTCCATAG AAAAGGTATC AGTCAGAGGT TCAGTCTCCA CCCTGGAAGC CGGACCTTCG GATACCTGTC CTATGGACAG	CAATTAATTC TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC CAAAAGGCCA GTTTTCCGGT GCTCCGCCCC CGAGGCGGGG GCCAAACCC CCGCTTTGGG TCCCTCGTGC AGGGAGCACG

Figure 27 AK

35051		CCGCTGCGCC GGCGACGCGG		
35101		ACGACTTATC TGCTGAATAG	 	
35151		AGGTATGTAG TCCATACATC		
35201		CTACACTAGA GATGTGATCT		
35251		CCTTCGGAAA GGAAGCCTTT		
35301		GGTAGCGGTG CCATCGCCAC	 	
35351	*	AGGATCTCAA TCCTAGAGTT	 	
35401		GGAACGAAAA CCTTGCTTTT		
35451		ATCTTCACCT TAGAAGTGGA	 	
35501		TGGTCTGACA ACCAGACTGT	 	
35551		CTGTCTATTT GACAGATAAA		
35601		CTACGATACG GATGCTATGC	 	
35651		CGAGACCCAC GCTCTGGGTG	 	
35701		CGGAAGGGCC GCCTTCCCGG	 	
35751		AGTCTATTAA TCAGATAATT		TAAGTAGTTC ATTCATCAAG
35801		AGTTTGCGCA TCAAACGCGT		
35851		GTCGTTTGGT CAGCAAACCA		TTCCCAACGA AAGGGTTGCT
35901		TTACATGATC AATGTACTAG		
35951		CCGATCGTTG GGCTAGCAAC	 	GTGTTATCAC CACAATAGTG

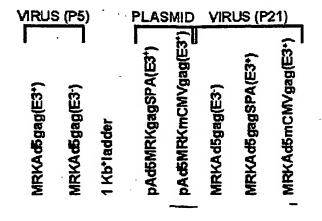
Figure 27AL

WO 02/2	2080					PCT/US01/28861
36001	TCATGGTTAT AGTACCAATA	AGCACTG CCGTCGTGAC	CATAATTCTC GTATTAAGAG	TTACTGTCAT AATGACAGTA	GCCATC TA CGGTAGGCAT	
36051	AGATGCTTTT TCTACGAAAA		TGAGTACTCA ACTCATGAGT			
36101	GTGTATGCGG CACATACGCC		GCTCTTGCCC CGAGAACGGG			
36151	CCGCGCCACA GGCGCGGTGT	TAGCAGAACT ATCGTCTTGA	TTAAAAGTGC AATTTTCACG	TCATCATTGG AGTAGTAACC	AAAACGTTC:	7
36201	TCGGGGCGAA AGCCCCGCTT		GATCTTACCG CTAGAATGGC			
36251	GTAACCCACT CATTGGGTGA	CGTGCACCCA GCACGTGGGT	ACTGATCTTC TGACTAGAAG	AGCATCTTTT TCGTAGAAAA	ACTTTCACCA TGAAAGTGG	r
36301 -	GCGTTTCTGG CGCAAAGACC	GTGAGCAAAA CACTCGTTTT	ACAGGAAGGC TGTCCTTCCG	AAAATGCCGC TTTTACGGCG	AAAAAAGGGG	A F
36351	ATAAGGGCGA TATTCCCGCT		TTGAATACTC AACTTATGAG			
36401	TTATTGAAGC AATAACTTCG		GTTATTGTCT CAATAACAGA			
36451	AATGTATTTA TTACATAAAT		CAAATAGGGG GTTTATCCCC			
36501	AAAGTGCCAC TTTCACGGTG		AGAAACCATT TCTTTGGTAA			
36551	TAAAAATAGG ATTTTTATCC		GGCCCTTTCG CCGGGAAAGC			

PacI

36601 ATTCTTAATT TCTTAATTAA (SEQ ID NO:34) TAAGAATTAA AGAATTAATT (SEQ ID NO:35)

Figure 27AM



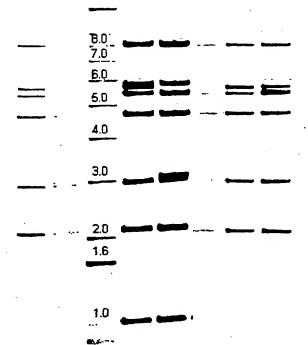


FIGURE 28

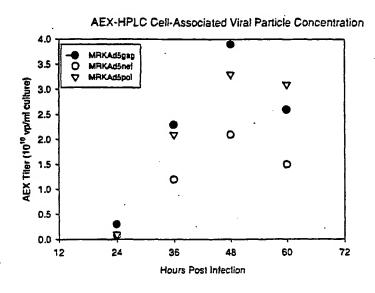


FIGURE 29A

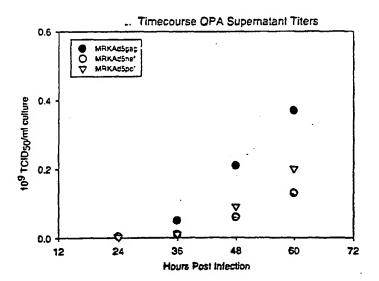


FIGURE 29B

			atg Met													48
			gtt Val 20													96
			agg Arg													144
			cag Gln													192
			ctg Leu													240
			aag Lys													288
			gag Glu 100													336
			ggc Gly													384
			cag Gln													432
ctg Leu 145	aat Asn	gcc Ala	tgg Trp	gtg Val	aag Lys 150	gtg Val	gtg Val	gag Glu	gag Glu	aag Lys 155	gcc Ala	ttc Phe	tcc Ser	cct Pro	gag Glu 160	480
			atg Met													528
ctg Leu	aac Asn	acc Thr	atg Met 180	ctg Leu	aac Asn	aca Thr	gtg Val	ggg Gly 185	ggc Gly	cat His	cag Gln	gct Ala	gcc Ala 190	atg Met	cag Gln	576
			gag Glu													624
			cac His													672
agg Arg 225	ggc Gly	tct Ser	gac Asp	att Ile	gct Ala 230	ggc Gly	acc Thr	acc Thr	tcc Ser	acc Thr 235	ctc Leu	cag Gln	gag Glu	cag Gln	att Ile 240	720
ggc Gly	tgg Trp	atg Met	acc Thr	aac Asn 245	aac Asn	ccc Pro	ccc Pro	atc Ile	cct Pro 250	gtg Val	Gly	gaa Glu	atc Ile	tac Tyr 255	aag Lys	768

Figure 30'A°

agg Arg	tgg Trp	atc Ile	atc Ile 260	ctg Leu	ggc ggc	ctg Leu	aac Asn	aag Lys 265	att Ile	gtg Val	agg Arg	atg Met	tac Tyr 270	tcc Ser	ccc Pro	816
acc Thr	tcc Ser	atc Ile 275	ctg Leu	gac Asp	atc Ile	agg Arg	cag Gln 280	ggc Gly	ccc Pro	aag Lys	gag Glu	ccc Pro 285	ttc Phe	agg Arg	gac Asp	864
tat Tyr	gtg Val 290	gac Asp	agg Arg	ttc Phe	tac Tyr	aag Lys 295	acc Thr	ctg Leu	agg Arg	gct Ala	gag Glu 300	cag Gln	gcc Ala	tcc Ser	cag Gln	912
gag Glu 305	gtg Val	aag Lys	aac Asn	tgg Trp	atg Met 310	aca Thr	gag Glu	acc Thr	ctg Leu	ctg Leu 315	gtg Val	cag Gln	aat Asn	gcc Ala	aac Asn 320	960
cct Pro	gac Asp	tgc Cys	aag Lys	acc Thr 325	atc Ile	ctg Leu	aag Lys	gcc Ala	ctg Leu 330	ggc Gly	cct Pro	gct Ala	gcc Ala	acc Thr 335	ctg Leu	1008
gag Glu	gag Glu	atg Met	atg Met 340	aca Thr	gcc Ala	tgc Cys	cag Gln	ggg Gly 345	gtg Val	GJA āāā	ggc Gly	cct Pro	ggt Gly 350	cac His	aag Lys	1056
gcc Ala	agg Arg	gtg Val 355	ctg Leu	gct Ala	gag Glu	gcc Ala	atg Met 360	tcc Ser	cag Gln	gtg Val	acc Thr	aac Asn 365	tcc Ser	gcc Ala	acc Thr	1104
atc Ile	atg Met 370	atg Met	cag Gln	agg Arg	ggc Gly	aac Asn 375	ttc Phe	agg Arg	aac Asn	cag Gln	agg Arg 380	aag Lys	aca Thr	gtg Val	aag Lys	1152
tgc Cys 385	Phe	aac Asn	tgt Cys	Gly	aag Lys 390	gtg Val	ggc Gly	cac His	att Ile	gcc Ala 395	aag Lys	aac Asn	tgt Cys	agg Arg	gcc Ala 400	1200
ccc Pro	agg Arg	aag Lys	aag Lys	ggc Gly 405	tgc Cys	tgg [.] Trp	aag Lys	tgt Cys	ggc Gly 410	aag Lys	gag Glu	ggc Gly	cac His	cag Gln 415	atg Met	1248
aag Lys	gac Asp	tgc Cys	aat Asn 420	gag Glu	agg Arg	cag Gln	gcc Ala	aac Asn 425	ttc Phe	ctg Leu	ggc Gly	aaa Lys	atc Ile 430	tgg Trp	ccc Pro	1296
tcc Ser	cac His	aag Lys 435	ggc Gly	agg Arg	cct Pro	Gly	aac Asn 440	ttc Phe	ctc Leu	cag Gln	tcc Ser	agg Arg 445	cct Pro	gag Glu	ecc Pro	1344
aca Thr	gcc Ala 450	cct Pro	ccc Pro	gag Glu	gag Glu	tcc Ser 455	ttc Phe	agg Arg	ttť Phe	Gly ggg	gag Glu 460	gag Glu	aag Lys	acc Thr	acc Thr	1392
Pro 465	Ser	Gln	aag Lys	Gln	Glu 470	Pro	Ile	Asp	Lys	475	Leu	туг	Pro	ren	480	1440
tcc Ser	ctg Leu	agg Arg	tcc Ser	ctg Leu 485	ttt Phe	ggc	aac Asn	gac Asp	Pro 490	tcc Ser	tcc Ser	cag Gln	taa *	(SI	D NO:36) D NO:37)	1482

Figure 30 B

Figure 31

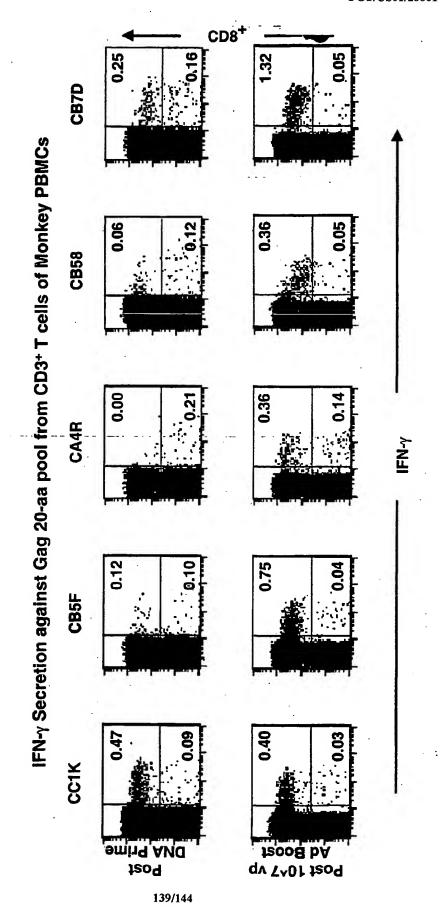


FIGURE 32

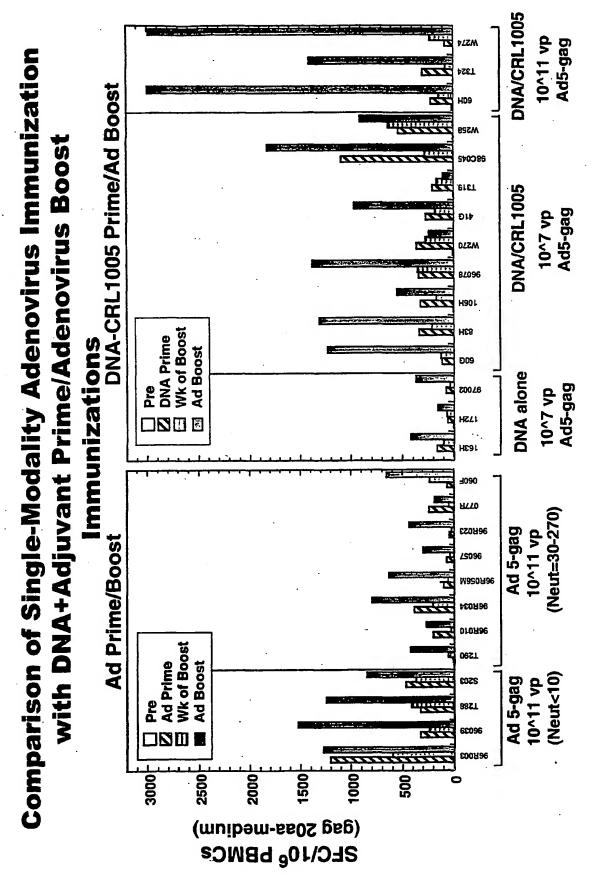


FIGURE 33A

ATGGGTGCTA	GGGCTTCTGT	GCTGTCTGGT	GGTGAGCTGG	ACAAGTGGGA	GAAGATCAGG
CTGAGGCCTG	GTGGCAAGAA	GAAGTACAAG	CTAAAGCACA	TTGTGTGGGC	CTCCAGGGAG
CTGGAGAGGT	TTGCTGTGAA	CCCTGGCCTG	CTGGAGACCT	CTGAGGGGTG	CAGGCAGATC
CTGGGCCAGC	TCCAGCCCTC	CCTGCAAACA	GGCTCTGAGG	AGCTGAGGTC	CCTGTACAAC
ACAGTGGCTA	CCCTGTACTG	TGTGCACCAG	AAGATTGATG	TGAAGGACAC	CAAGGAGGCC
CTGGAGAAGA	TTGAGGAGGA	GCAGAACAAG	TCCAAGAAGA	AGGCCCAGCA	GGCTGCTGCT
GGCACAGGCA	ACTCCAGCCA	GGTGTCCCAG	AACTACCCCA	TTGTGCAGAA	CCTCCAGGGC
CAGATGGTGC	ACCAGGCCAT	CTCCCCCGG	ACCCTGAATG	CCTGGGTGAA	GGTGGTGGAG
GAGAAGGCCT	TCTCCCCTGA	GGTGATCCCC	ATGTTCTCTG	${\tt CCCTGTCTGA}$	GGGTGCCACC
CCCCAGGACC	TGAACACCAT	GCTGAACACA	GTGGGGGGCC	ATCAGGCTGC	CATGCAGATG
CTGAAGGAGA	CCATCAATGA	GGAGGCTGCT	GAGTGGGACA	GGCTGCATCC	TGTGCACGCT
GGCCCCATTG	CCCCGGCCA	GATGAGGGAG	CCCAGGGGCT	${\tt CTGACATTGC}$	TGGCACCACC
TCCACCCTCC	AGGAGCAGAT	${\tt TGGCTGGATG}$	ACCAACAACC	CCCCCATCCC	TGTGGGGGAA
ATCTACAAGA	GGTGGATCAT	CCTGGGCCTG	AACAAGATTG	TGAGGATGTA	CTCCCCCACC
TCCATCCTGG	ACATCAGGCA	GGGCCCCAAG	GAGCCCTTCA	GGGACTATGT	GGACAGGTTC
TACAAGACCC	TGAGGGCTGA	GCAGGCCTCC	CAGGAGGTGA	AGAACTGGAT	GACAGAGACC
CTGCTGGTGC	AGAATGCCAA	CCCTGACTGC	AAGACCATCC	TGAAGGCCCT	GGGCCCTGCT
GCCACCCTGG	AGGAGATGAT	GACAGCCTGC	CAGGGGGTGG	GGGGCCCTGG	TCACAAGGCC
AGGGTGCTGG	CTGAGGCCAT	GTCCCAGGTG	ACCAACTCCG	CCACCATCAT	GATGCAGAGG
GGCAACTTCA	GGAACCAGAG	GAAGACAGTG	AAGTGCTTCA	ACTGTGGCAA	GGTGGGCCAC
ATTGCCAAGA	ACTGTAGGGC	CCCCAGGAAG	AAGGGCTGCT	GGAAGTGTGG	CAAGGAGGGC
CACCAGATGA	AGGACTGCAA	TGAGAGGCAG	GCCAACTTCC	TGGGCAAAAT	CTGGCCCTCC
CACAAGGGCA	GGCCTGGCAA	CTTCCTCCAG	TCCAGGCCTG	AGCCCACAGC	CCCTCCCGAG
GAGTCCTTCA	GGTTTGGGGA	GGAGAAGACC	ACCCCCAGCC	AGAAGCAGGA	GCCCATTGAC
AAGGAGCTGT	ACCCCCTGGC	CTCCCTGAGG	TCCCTGTTTG	GCAACGACCC	CTCCTCCCAG
ATGGCTCCCA	TCTCCCCCAT	TGAGACTGTG	CCTGTGAAGC	TGAAGCCTGG	CATGGATGGC
CCCAAGGTGA	AGCAGTGGCC	CCTGACTGAG	GAGAAGATCA	AGGCCCTGGT	GGAAATCTGC
ACTGAGATGG	AGAAGGAGGG	CAAAATCTCC	AAGATTGGCC	CCGAGAACCC	CTACAACACC
CCTGTGTTTG	CCATCAAGAA	GAAGGACTCC	ACCAAGTGGA	GGAAGCTGGT	GGACTTCAGG
GAGCTGAACA	AGAGGACCCA	GGACTTCTGG	GAGGTGCAGC	TGGGCATCCC	CCACCCCGCT
GGCCTGAAGA	AGAAGAAGTC	TGTGACTGTG	CTGGCTGTGG	GGGATGCCTA	CTTCTCTGTG
CCCCTGGATG	AGGACTTCAG	GAAGTACACT	GCCTTCACCA	TCCCCTCCAT	CAACAATGAG
ACCCCTGGCA	TCAGGTACCA	GTACAATGTG	CTGCCCCAGG	GCTGGAAGGG	CTCCCCTGCC
ATCTTCCAGT	CCTCCATGAC	CAAGATCCTG	GAGCCCTTCA	GGAAGCAGAA	CCCTGACATT
GTGATCTACC	AGTACATGGC	TGCCCTGTAT	GTGGGCTCTG	ACCTGGAGAT	TGGGCAGCAC
AGGACCAAGA	TTGAGGAGCT	GAGGCAGCAC	CTGCTGAGGT	GGGGCCTGAC	CACCCCTGAC
AAGAAGCACC	AGAAGGAGCC	CCCCTTCCTG	TGGATGGGCT	ATGAGCTGCA	CCCCGACAAG
TGGACTGTGC	AGCCCATTGT	GCTGCCTGAG	AAGGACTCCT	GGACTGTGAA	TGACATCCAG
AAGCTGGTGG	GCAAGCTGAA	CTGGGCCTCC	CAAATCTACC	CTGGCATCAA	GGTGAGGCAG
CTGTGCAAGC	TGCTGAGGGG	CACCAAGGCC	CTGACTGAGG	TGATCCCCCT	GACTGAGGAG
GCTGAGCTGG	AGCTGGCTGA	GAACAGGGAG	ATCCTGAAGG	AGCCTGTGCA	TGGGGTGTAC

FIGURE 33B

TATGACCCCT	CCAAGGACCT	GATTGCTGAG	ATCCAGAAGC	AGGGCCAGGG	CCAGTGGACC
TACCAAATCT	ACCAGGAGCC	CTTCAAGAAC	CTGAAGACTG	GCAAGTATGC	CAGGATGAGG
GGGGCCCACA	CCAATGATGT	GAAGCAGCTG	ACTGAGGCTG	TGCAGAAGAT	CACCACTGAG
TCCATTGTGA	TCTGGGGCAA	GACCCCCAAG	${\tt TTCAAGCTGC}$	CCATCCAGAA	GGAGACCTGG
GAGACCTGGT	GGACTGAGTA	CTGGCAGGCC	ACCTGGATCC	CTGAGTGGGA	GTTTGTGAAC
ACCCCCCCC	TGGTGAAGCT	GTGGTACCAG	CTGGAGAAGG	AGCCCATTGT	GGGGGCTGAG
ACCTTCTATG	TGGCTGGGGC	TGCCAACAGG	GAGACCAAGC	TGGGCAAGGC	TGGCTATGTG
ACCAACAGGG	GCAGGCAGAA	GGTGGTGACC	CTGACTGACA	CCACCAACCA	GAAGACTGCC
CTCCAGGCCA	TCTACCTGGC	CCTCCAGGAC	TCTGGCCTGG	AGGTGAACAT	TGTGACTGCC
TCCCAGTATG	CCCTGGGCAT	CATCCAGGCC	CAGCCTGATC	AGTCTGAGTC	TGAGCTGGTG
AACCAGATCA	TTGAGCAGCT	GATCAAGAAG	GAGAAGGTGT	ACCTGGCCTG	GGTGCCTGCC
CACAAGGGCA	TTGGGGGCAA	TGAGCAGGTG	GACAAGCTGG	TGTCTGCTGG	CATCAGGAAG
GTGCTGTTCC	TGGATGGCAT	TGACAAGGCC	CAGGATGAGC	ATGAGAAGTA	CCACTCCAAC
TGGAGGGCTA	TGGCCTCTGA	CTTCAACCTG	CCCCTGTGG	TGGCTAAGGA	GATTGTGGCC
TCCTGTGACA	AGTGCCAGCT	GAAGGGGGAG	GCCATGCATG	GGCAGGTGGA	CTGCTCCCCT
GGCATCTGGC	AGCTGGCCTG	CACCCACCTG	GAGGGCAAGG	TGATCCTGGT	GGCTGTGCAT
GTGGCCTCCG	GCTACATTGA	GGCTGAGGTG	ATCCCTGCTG	AGACAGGCCA	GGAGACTGCC
TACTTCCTGC	TGAAGCTGGC	TGGCAGGTGG	CCTGTGAAGA	CCATCCACAC	TGCCAATGGC
TCCAACTTCA	CTGGGGCCAC	AGTGAGGGCT	GCCTGCTGGT	GGGCTGGCAT	CAAGCAGGAG
TTTGGCATCC	CCTACAACCC	CCAGTCCCAG	GGGGTGGTGG	CCTCCATGAA	CAAGGAGCTG
AAGAAGATCA	TTGGGCAGGT	GAGGGACCAG	GCTGAGCACC	TGAAGACAGC	TGTGCAGATG
GCTGTGTTCA	TCCACAACTT	CAAGAGGAAG	GGGGGCATCG	GGGGCTACTC	CGCTGGGGAG
AGGATTGTGG	ACATCATTGC	CACAGACATC	CAGACCAAGG	AGCTCCAGAA	GCAGATCACC
AAGATCCAGA	ACTTCAGGGT	GTACTACAGG	GACTCCAGGA	ACCCCCTGTG	GAAGGGCCCT
GCCAAGCTGC	TGTGGAAGGG	GGAGGGGGCT	GTGGTGATCC	AGGACAACTC	TGACATCAAG
GTGGTGCCCA	GGAGGAAGGC	CAAGATCATC	AGGGACTATG	GCAAGCAGAT	GGCTGGGGAT
GACTGTGTGG	CCTCCAGGCA	GGATGAGGAC	TAA .		
SEQ ID NO:	38				

FIGURE 34A

Met Gly Ala Arg Ala Ser Val Leu Ser Gly Gly Glu Leu Asp Lys Trp Glu Lys Ile Arg Leu Arg Pro Gly Gly Lys Lys Lys Tyr Lys Leu Lys His Ile Val Trp Ala Ser Arg Glu Leu Glu Arg Phe Ala Val Asn Pro Gly Leu Leu Glu Thr Ser Glu Gly Cys Arg Gln Ile Leu Gly Gln Leu Gln Pro Ser Leu Gln Thr Gly Ser Glu Glu Leu Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys Val His Gln Lys Ile Asp Val Lys Asp Thr Lys Glu Ala Leu Glu Lys Ile Glu Glu Glu Gln Asn Lys Ser Lys Lys Lys Ala Gln Gln Ala Ala Gly Thr Gly Asn Ser Ser Gln Val Ser Gln Asn Tyr Pro Ile Val Gln Asn Leu Gln Gly Gln Met Val His Gln Ala Ile Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val Val Glu Glu Lys Ala Phe Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln Ala Ala Met Gln Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg Leu His Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln Met Arg Glu Pro Arg Gly Ser Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile Gly Trp Met Thr Asn Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu Asn Lys Ile Val Arg Met Tyr Ser Pro Thr Ser Ile Leu Asp Ile Arg Gln Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala Glu Gln Ala Ser Gln Glu Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val Gln Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala Ala Thr Leu Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly Pro Gly His Lys Ala Arg Val Leu Ala Glu Ala Met Ser Gln Val Thr Asn Ser Ala Thr Ile Met Met Gln Arg Gly Asn Phe Arg Asn Gln Arg Lys Thr Val Lys Cys Phe Asn Cys Gly Lys Val Gly His Ile Ala Lys Asn Cys Arg Ala Pro Arg Lys Lys Gly Cys Trp Lys Cys Gly Lys Glu Gly His Gln Met Lys Asp Cys Asn Glu Arg Gln Ala Asn Phe Leu Gly Lys Ile Trp Pro Ser His Lys Gly Arg Pro Gly Asn Phe Leu Gln Ser Arg Pro Glu Pro Thr Ala Pro Pro Glu Glu Ser Phe Arg Phe Gly Glu Glu Lys Thr Thr Pro Ser Gln Lys Gln Glu Pro Ile Asp Lys Glu Leu Tyr Pro Leu Ala Ser Leu Arg Ser Leu Phe Gly Asn Asp Pro Ser Ser Gln Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Ser Val Thr Val Leu Ala Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro

FIGURE 34B

Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Cly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly Jle Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp SEQ ID NO: 39

(19) World Intellectual Property Organization International Bureau



(43) International Publication Date 21 March 2002 (21.03.2002)

PCT

(10) International Publication Number WO 02/22080 A3

- (51) International Patent Classification7:

C12N 15/86

- (21) International Application Number: PCT/US01/28861
- (22) International Filing Date:

14 September 2001 (14.09.2001)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

60/233,180

15 September 2000 (15.09.2000)

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- (81) Designated States (national): AE. AG, AL. AM, AT, AU. AZ. BA, BB. BG. BR. BY. BZ. CA. CH. CN. CO, CR. CU. CZ. DE. DK. DM, DZ. EC. EE. ES. FI. GB. GD. GE. GH. GM. HR. HU, ID. IL. IN. IS. JP. KE. KG, KR, KZ. LC, LK. LR. LS. LT. LU. LV. MA. MD. MG, MK. MN. MW, MX, MZ. NO, NZ. PH. PL. PT. RO. RU. SD. SE, SG, SI, SK. SL. TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT. LU. MC. NL. PT. SE, TR), OAPI patent (BF, BJ, CF, CG. CI. CM, GA. GN. GQ, GW, ML, MR, NE, SN, TD. TG).

Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments
- (88) Date of publication of the international search report: 2 May 2002

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIVI-GAG. POL. NEF AND MODIFICATIONS

(57) Abstract: First generation adenoviral vectors and associated recombinant adenovirus-based HIV vaccines which show enhanced stability and growth properties and greater cellular-mediated immunity are described within this specification. These adenoviral vectors are utilized to generate and produce through cell culture various adenoviral-based HIV-1 vaccines which contain HIV-I gag, HIV-I pol and/or HIV-I nef polynucleotide pharmaceutical products, and biologically relevant modifications thereof. These adenovirus vaccines, when directly introduced into living vertebrate tissue, preferably a mammalian host such as a human or a non-human mammal of commercial or domestic veterinary importance, express the HIV1- Gag. Pol and/or Ncf protein or biologically modification thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding HIV-1 Gag, encoding codon optimized HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNAse H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef. such as myristylation and down regulation of host CD4. The adenoviral vaccines of the present invention, when administered alone or in a combined modality regime, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HtV-1 infection.

International application No.

PCT/US01/28861

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	SIFICATION OF SUBJECT MATTER		·			
IPC(7)	: C12N 15/86					
US CL	US CL: 435/456 According to International Patent Classification (IPC) or to both national classification and IPC					
	International Patent Classification (If C) of the source					
	OS SEARCHED					
Minimum doc	numentation searched (classification system followed b	y classification symbols)				
U.S. : 42	24/205.1, 207.1, 227.1, 233.1; 435/69.1, 69.3, 173.3,	, 235.1, 320.1, 456; 530/23.72;				
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Documentatio	on searched other than minimum documentation to the	EXICIN CIRC SUCH COCCUMENTS INC BISTORY				
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		of data have and where practicable. S	earch terms used)			
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Please See Co	ontinuation Sheet		i			
c poct	UMENTS CONSIDERED TO BE RELEVANT					
	Citation of document, with indication, where app	propriate, of the relevant passages	Relevant to claim No.			
Category *	WO 96/39178 (ERTL et al.) 12 December 1996 (12.	12 1006) see page 5 6 10 12 13	1-3, 8-11, 18			
Х		12.1770), 300 page 3, 0,10, 12, 12				
	and claims 1 and 5.		4, 5, 13-17, 29, 30,			
Y		i	32, 34, 35, 37			
	US 6,019,978 A (ERTL et al.) 1 February 2000 (01/	(02/2000) see columns 2 7 and 8	1-3, 8-11, 18			
Х	US 6,019,978 A (ERIL et al.) 1 February 2000 (01)	022000), see column 2, , ale co				
			4, 5, 13-17, 29, 30,			
Y			32, 34, 35, 37			
	US 6,287,571 A A (ERTL et al.) 11 September 2001	(11/00/2001) see columns 2 7 8	1, 9, 18			
X,P		(11/03/2001); see columns 2; 7; 5				
	and claim 1. US 5,643,579A (HUNG et al.) 1 July 1997 (01/07/1	007) see examples 1 2 25 and 26.	1-3, 8, 9-11, 18			
X	US 5,643,579A (HUNG et al.) 1 July 1997 (01/07/1	997), see champles 1, 2, 25 and 20.				
	1		4,5,13-17, 29, 30, 32,			
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	S. Et latest resiliention	defection adenovirus recombinant	1-3, 9-11, 13-18			
Y	WANG et al. The use of an E1-deleted, replication	-delective adelity if us recombinant	1 20,7 1., 10 10			
expressing the rabies virus glycoprotein for early vaccination of mice against rabies virus.						
	Journal of Virology (March 1997) Vol. 71, No. 5, p	p 3017-3063.	i ·			
	<u> </u>					
Furthe	er documents are listed in the continuation of Box C.	See patent family annex.				
	Special categories of cited documents:	"T" later document published after the int	emational filing date or priority			
ł		date and not in conflict with the appli principle or theory underlying the inv	cation but cited to understand the			
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Date of the	actual completion of the international search	S SAND 2001	шингерин			
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	Commissioner of Patents and Trademarks Box PCT Ulrike Winkler, Ph.D.					
	ox PC 1 /ashington, D.C. 20231	700 000 000	Vollens)			
1	No. (703)305-3230	Telephone No. 703-308-0196	Y			
1	SA/210 (second sheet) (July 1998)					
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International application No.

PCT/US01/28861

INTERNATIONAL SEARCH REPORT

ategory *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NATUK et al. Immunogenicity of recombinant human adenovirus -human immunodeficiency virus vaccines in chimpanzees. Aids Research and Human Retroviruses (1993) Vol. 9, No. 5, pp395-404, see material and methods.	1, 9, 29, 30, 32
Y	PREVEC et al. Immune response to HIV-1 gag antigens induced by recombinant adenovirus vectors in mice and rhesus macaque monkeys. Journal of Acquired Immune Deficincy Syndrome. (1991) Vol. 4, No. 6 pp. 568-76, see abstract.	1, 9, 29, 30, 32
Y	LORI et al. Rapid protection against human immunodeficiency virus type 1 (HIV-1) replication mediated by high efficiency non-retroviral delivery of genes interfering with HIV-1 tat and gag. Gene Therapy (1994) Vol. 1, No. 1, pp. 27-31, see abstract.	1, 9
Y	PFARR et al. Differential effects of polyadenylation regions on gene expression in mammalian cells. DNA (1986) Vol. 5, No. 2, pp.115-22, see abstract.	. 16
Y	NATUK et al. Adenovirus vectored vaccine. Developmental Biological Standards (1994) Vol. 82, pp. 71-77, see abstract.	1, 9
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International application No.

PCT/US01/28861

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)				
This international report has not been established in respect of certain claims under Article 17(2)(n) for the following reasons:				
Claim Nos.: because they relate to subject matter not required to be searched by this Authority, namely:				
2. Claim Nos.: 31 because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically: This claim could not be searched because applicant did not provide a CRF.				
Claim Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).				
Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)				
This International Searching Authority found multiple inventions in this international application, as follows: Please See Continuation Sheet				
1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims. 2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee. 3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:				
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-5, 8-11, 13-18, 29-32, 34, 35, 37 Remark on Protest The additional search fees were accompanied by the applicant's protest. No protest accompanied the payment of additional search fees.				

Form PCT/ISA/210 (continuation of first sheet(1)) (July 1998)

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The special technical feature of group 4, 16 and 31 is considered to be a method of producing recombinant adenoviral particles. Each group contains different sequences hence the resulting particles would have different structures and functions associated with the particle.

The special technical feature of group 5, 17 and 32 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors. Each group contains different sequences a encoding different protein, therefore the resulting immune response will also be different.

The special technical feature of group 6, 18 and 33 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors in conjunction with immunizing the individual a DNA plasmid vaccine. Each method contains different sequences encoding a different protein, therefore the resulting immune response will also be different.

Accordingly, groups 1-48 are not so linked by the same or corresponding technical feature as to form a single general inventive concept.

Continuation of B. FIELDS SEARCHED Item 3: WEST 2.0, STN-BIOSIS, MEDLINE

adenoviral vector, deletion, HIV, Gag, polyadenylation signal, CMV promoter

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		and ΔE3, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1)
4	55	inserted in E1. The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$
		and ΔE3, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in E1.
15	55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in E1.
16	57-61	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Pol protein.
17	62, 65, 66	The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle.
18	63, 64	The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.
19	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of <u>\Delta 1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in the parallel orientation of E1.
20	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of ΔΕ1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in the parallel orientation of Ε1.
21	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of <u>AE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in the parallel orientation of E1.
22	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of \$\Delta E1\$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in the parallel orientation of E1.
23	71	The claim is directed to an adenoviral vector that is at least partially deleted of <u>AE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 9)</u> inserted in the antiparallel orientation of E1.
24	71	The claim is directed to an adenoviral vector that is at least partially deleted of <u>AE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 11)</u> inserted in the antiparallel orientation of E1.
25	71	The claim is directed to an adenoviral vector that is at least partially deleted of ΔEI , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in the antiparallel orientation of E1.
26	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in the antiparallel orientation of E1.
27	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\triangle E1$ and $\triangle E3$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in E1.
28	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in E1.
29	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type

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		and ΔΕ3, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in E1.
14	55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in E1.
15	55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7)
16	57-61	inserted in E1. The claims are directed to a method of making and harvesting of a recombinant
17	62, 65, 66	adenoviral particle that contains a gene encoding an HIV Pol protein. The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle.
18	63, 64	The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.
19	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of <u>AE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in the parallel orientation of E1.
20	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of <u>AE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in the parallel orientation of E1.
21	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of <u>AE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in the parallel orientation of E1.
22	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of <u>AE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 15)</u> inserted in the parallel orientation of E1.
23	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in the antiparallel orientation of E1.
24	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in the antiparallel orientation of E1.
25	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in the antiparallel orientation of E1.
26	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in the antiparallel orientation of E1.
27	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in E1.
28	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in E1.
29	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type

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	T	adenovirus genome, and a gene which encodes an HIV Nel protein (SEQ ID NO: 13)
		inserted in E1. The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$
30	74	and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in E1
	76-80	The claims are directed to a method of making and harvesting of a recombinant
31	76-80	adenoviral particle that contains a gene encoding an HIV Nel protein.
32	81, 84, 85	The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle.
	82, 83	The claims are directed to a method of generating a cellular mediated immune
33	82, 83	response to HIV Nef with the recombinant adenoviral particle in addition to
	86a	The claim is drawn to a multivalent vaccine wherein gag, pol and nef are expressed
34	908	from three individual vectors.
35	86b, 88, 89	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed
	25 89	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed
36	86c, 88	from two individual vectors, one expressing nef-pol fusion and one expressing gag.
	86d, 87, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nej are expressed
37	800, 87, 88	from two individual vectors, one expressing gag-pol fusion and one expressing nej.
	86e, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed
38	000, 00	from two individual vectors, one expressing nef-gag fusion and one expressing pol.
39	86f, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from a single vectors as a fusion protein.
		The claims are drawn to a multivalent vaccine wherein gag and pol are expressed
40	86g, 88	from two individual vectors.
41	86h, 88, 89	The claims are drawn to a multivalent vaccine wherein gag and pol are expressed
41		individually from one vector.
42	86i, 88	The claims are drawn to a multivalent vaccine wherein pol and nef are expressed
42		from two individual vectors.
43	86j, 88, 89	The claims are drawn to a multivalent vaccine wherein pol and nef are expressed
13		from individually from one vector.
44	86k, 88	The claims are drawn to a multivalent vaccine wherein nef and gag are expressed
		individually from one vector.
45	861, 88, 89	The claims are drawn to a multivalent vaccine wherein nef and gug are expressed individually from one vector.
46	86m, 88	The claims are drawn to a multivalent vaccine wherein gag and pol are expressed as fusion protein from one vector.
12	86n, 88	The claims are drawn to a multivalent vaccine wherein pol and nef are expressed as
47	8011, 00	fusion protein from one vector.
48	86u, 88	The claims are drawn to a multivalent vaccine wherein nef and gag are expressed as
} "	000, 00	fusion protein from one vector.

The inventions listed as Groups 1-48 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The technical feature linking groups 1-33 appears to be a recombinant adenoviral vector wherein the adenoviral vector is at least partially deleted in E1 but the vector may contain more deletions, the vector contains wild type sequences including packaging signals and a gene encoding a heterologous HIV protein or fragments thereof. Ertl et al. (WO 96/39178) disclose a recombinant adenoviral vector that is deleted in E1 and partially deleted in E3, the remainder of the adenoviral vector contains wild type sequences. The vector additionally contains an insertion of a heterologous protein which includes HIV proteins (see abstract and claims 1 and 5). Therefore, the technical feature linking the inventions of groups 1-45 does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art.

The special technical feature of the following groups 1-3, 7-15, 19-30 and 34-48 is considered to be the combination of sequences that is disclosed in each group, see individual claim groupings above for the different sequences. The DNA disclosed in each group is trade up of a different sequence having a different structure and different function.

REVISED VERSION

(19) World Intellectual Property Organization International Bureau





(43) International Publication Date 21 March 2002 (21.03.2002)

(10) International Publication Number WO 02/022080 A3

(51) International Patent Classification7:

- (21) International Application Number: PCT/US01/28861
- (22) International Filing Date:

14 September 2001 (14.09.2001)

(25) Filing Language:

English

C12N 15/86

(26) Publication Language:

English

(30) Priority Data:

60/233,180

15 September 2000 (15.09.2000)

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- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, 7.W
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- (88) Date of publication of the international search report: 2 May 2002 Date of publication of the revised international search report: 16 January 2003
- (15) Information about Corrections:

see PCT Gazette No. 03/2003 of 16 January 2003, Section II

Previous Correction:

see PCT Gazette No. 30/2002 of 25 July 2002, Section II

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS

(57) Abstract: First generation adenoviral vectors and associated recombinant adenovirus-based HIV vaccines which show enhanced stability and growth properties and greater cellular-mediated immunity are described within this specification. These adenoviral vectors are utilized to generate and produce through cell culture various adenoviral-based HIV-1 vaccines which contain HIV-1 gag, HIV-1 pol and/or HIV-1 nef polynucleotide pharmaceutical products, and biologically relevant modifications thereof. These adenovirus vaccines, when directly introduced into living vertebrate tissue, preferably a mammalian host such as a human or a non-human mammal of commercial or domestic veterinary importance, express the HIV1- Gag, Pol and/or Nef protein or biologically modification thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding HIV-1 Gag, encoding codon optimized HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNAse H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The adenoviral vaccines of the present invention, when administered alone or in a combined modality regime, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.

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		FC170301/2	0001		
	A. CLASSIFICATION OF SUBJECT MATTER IPC(7) : C12N 15/86				
US CL	: 435/456				
	International Patent Classification (IPC) or to both n	ational classification and IPC			
	DS SEARCHED	adolan Grassmonian and H C			
Minimum do	cumentation searched (classification custom followed	by election symbols			
	Minimum documentation searched (classification system followed by classification symbols) U.S.: 424/205.1, 207.1, 227.1, 233.1; 435/69.1, 69.3, 173.3, 235.1, 320.1, 456; 530/23.72;				
Documentation	on searched other than minimum documentation to the	e extent that such documents are inc	luded in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Please See Continuation Sheet					
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	UMENTS CONSIDERED TO BE RELEVANT	-1	I man and a second		
Category *	Citation of document, with indication, where ap		Relevant to claim No.		
X	WO 96/39178 (ERTL et al.) 12 December 1996 (12 and claims 1 and 5.	.12.1996), see page 5, 6,10, 12, 13	1-3, 8-11, 18		
Y	ant cams I and J.		4, 5, 13-17, 29-32, 34, 35, 37		
<u>x</u>	US 6,019,978 A (ERTL et al.) 1 February 2000, (01	/02/2000), see columns 2, 7 and 8.	1-3, 8-11, 18		
Y			4, 5, 13-17, 29-32, 34, 35, 37		
X,P	US 6,287,571 6 (ERTL et al.) 11 September 200 and claim 1.	1 (11/09/2001), see columns 2, 7, 8	1, 9, 18		
<u>x</u>	US 5,643,579A (HUNG et al.) 1 July 1997 (01/07/1997), see examples 1, 2, 25 and 26.				
Y			4,5,13-17, 29-32, 34, 35, 37		
Y WANG et al. The use of an E1-deleted, replication - expressing the rabies virus glycoprotein for early vac Journal of Virology (March 1997) Vol. 71, No. 5, pp		accination of mice against rabies vir	us. 1-3, 9-11, 13-18		
		·	·		
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	documents are listed in the continuation of Box C.	See patent family amex.			
* S	pecial categories of cited documents:		the international filing date or et with the application but cited to		
	t defining the general state of the art which is not considered to ticular relevance	understand the principle or the	cory underlying the invention		
"E" carlier ap					
"L" document which may throw doubts on priority claim(s) or which is cited "Y" document of particular relevance; the claimed invention cannot to establish the publication date of another citation or other special reason considered to involve an inventive step when the document is cumbined with one or more other such documents, such			ntive step when the document is ther such documents, such		
"O" documen					
	"A" document member of the same patent family "D" document published prior to the international filing date but later than the priority date claimed.				
Date of the a	Date of the actual completion of the international search Date of mailing of the international search report				
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Box	PCT	Ulrike Winkler, Ph.D.	~~~ \\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\		
	shington, D.C. 20231 o. (703)305-3230	Telephone No. 703-308-0196	1) \		
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Form PCT/ISA/210 (second sheet) (July 1998)

International application No.

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ategory •	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y .	NATUK et al. Immunogenicity of recombinant human adenovirus -human immunodeficiency virus vaccines in chimpanzees. Aids Research and Human Retroviruses (1993) Vol. 9, No. 5, pp395-404, see material and methods.	1, 9, 29-32
Y	PREVEC et al. Immune response to HIV-1 gag antigens induced by recombinant adenovirus vectors in mice and rhesus macaque monkeys. Journal of Acquired Immune Deficincy Syndrome. (1991) Vol. 4, No. 6 pp. 568-76, see abstract.	1, 9, 29-32
Y	LORI et al. Rapid protection against human immunodeficiency virus type 1 (HIV-1) replication mediated by high efficiency non-retroviral delivery of genes interfering with HIV-1 tat and gag. Gene Therapy (1994) Vol. 1, No. 1, pp. 27-31, see abstract.	1,9
Y	PFARR et al. Differential effects of polyadenylation regions on gene expression in mammalian cells. DNA (1986) Vol. 5, No. 2, pp.115-22, see abstract.	16
Y	NATUK et al. Adenovirus vectored vaccine. Developmental Biological Standards (1994) Vol. 82, pp. 71-77, see abstract.	1,9
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International application No.

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Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)				
This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:				
Claim Nos.: because they relate to subject matter not required to be searched by this Authority, namely:				
Claim Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:				
3. Claim Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).				
Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)				
This International Searching Authority found multiple inventions in this international application, as follows: Please See Continuation Sheet				
 As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.: 				
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-5, 8-11, 13-18, 29-32, 34,				
Remark on Protest The additional search fees were accompanied by the applicant's protest. No protest accompanied the payment of additional search fees.				

Form PCT/ISA/210 (continuation of first sheet(1)) (July 1998)

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BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group	Claims	
1	1-5, 8-11, 13-18, 29, 30, 31, 32, 34, 35, 37	The claims are directed to an adenoviral vector that is at least partially deleted of <u>AEI</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Gag protein (SEQ ID NO: 29)</u> inserted in the <u>parallel orientation of E1</u> . In addition the vector contains a promoter and a polyadenylation signal.
2	6, 7, 36	The claims are directed to an adenoviral vector that is at least partially deleted of <u>AE1 and AE3</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Gag protein (SEQ ID NO: 29).
3	12, 33	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV protein inserted in the antiparallel orientation of E1.
4	19-23, 38-42	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Gag protein.
5	24, 27, 28, 43, 46, 47	The claim is directed to a method of generating a cellular mediated immune response to HIV Gag protein with the recombinant adenoviral particle.
6	25, 26, 44, 45	The claim is directed to a method of generating a cellular mediated immune response to HIV Gag protein with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.
7	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in the parallel orientation of E1.
8	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of <u>AEI</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 5)</u> inserted in the parallel orientation of E1.
9	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in the parallel orientation of E1.
10	52	The claim is directed to an adenoviral vector that is at least partially deleted of <u>AE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 1)</u> inserted in the antiparallel orientation of E1.
11	52	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in the antiparallel orientation of E1.
12	52	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE_1 , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in the antiparallel orientation of E1.
13	55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$

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		and ΔE3, the vector contains the cis-acting packaging sequence of the wild type	
		adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1)	
	-	inserted in E1.	
14	55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$	
14	33	and AE3, the vector contains the cis-acting packaging sequence of the wild type	
15		adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5)	
		inserted in E1.	
15	55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$	
		and AE3, the vector contains the cis-acting packaging sequence of the wild type	
		adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7)	
16		inserted in E1.	
	57-61	The claims are directed to a method of making and harvesting of a recombinant	
17		adenoviral particle that contains a gene encoding an HIV Pol protein.	
	62, 65, 66	The claim is directed to a method of generating a cellular mediated immune response	
		to HIV Pol protein with the recombinant adenoviral particle.	
18	63, 64	The claim is directed to a method of generating a cellular mediated immune response	
		to HIV Pol protein with the recombinant adenoviral particle in addition to	
		administering a DNA plasmid vaccine.	
19	67-70, 72,	The claims are directed to an adenoviral vector that is at least partially deleted of	
	73, 75	ΔE1, the vector contains the cis-acting packaging sequence of the wild type	
		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9)	
		inserted in the parallel orientation of E1.	
20	67-70, 72,	The claims are directed to an adenoviral vector that is at least partially deleted of	
	73, 75	ΔE1, the vector contains the cis-acting packaging sequence of the wild type	
	,	adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11)	
	ļ	inserted in the parallel orientation of E1.	
21 · ·	67-70, 72,	The claims are directed to an adenoviral vector that is at least partially deleted of	
21	73, 75	ΔE1, the vector contains the cis-acting packaging sequence of the wild type	
•	13, 13		
	1	adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13)	
22	67.70.70	inserted in the parallel orientation of E1.	
22	67-70, 72,	The claims are directed to an adenoviral vector that is at least partially deleted of	
	73, 75	ΔE_1 , the vector contains the cis-acting packaging sequence of the wild type	
	1	adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15)	
		inserted in the parallel orientation of E1.	
23	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$,	
	1	the vector contains the cis-acting packaging sequence of the wild type adenovirus	
		genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in	
		the antiparallel orientation of E1.	
24	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$,	
		the vector contains the cis-acting packaging sequence of the wild type adenovirus	
		genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in	
		the antiparallel orientation of E1.	
25	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$,	
		the vector contains the cis-acting packaging sequence of the wild type adenovirus	
]	genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in	
		the antiparallel orientation of E1.	
26	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$,	
		the vector contains the cis-acting packaging sequence of the wild type adenovirus	
		genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in	
		the antiparallel orientation of E1.	
27	74	The claim is directed to an adenoviral vector that is at least partially deleted of AE1	
	· ·	and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type	
	1	adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9)	
20		inserted in E1.	
28	74	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE_1	
	1	and AE3, the vector contains the cis-acting packaging sequence of the wild type	
		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11)	•
		inserted in E1.	
29	74	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE_1	
		and AE3, the vector contains the cis-acting packaging sequence of the wild type	

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		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in E1.	
30	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in E1.	
31	76-80	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Nef protein.	
32	81, 84, 85	The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle.	
33	82, 83	The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.	
34	86a	The claim is drawn to a multivalent vaccine wherein gag, pol and nef are expressed from three individual vectors.	
35	86b, 88, 89	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from one individual vectors.	
36	86c, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from two individual vectors, one expressing nef-pol fusion and one expressing gag.	
37	86d, 87, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from two individual vectors, one expressing gag-pol fusion and one expressing nef.	
38	86e, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from two individual vectors, one expressing nef-gag fusion and one expressing pol.	
39	86f, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from a single vectors as a fusion protein.	
40	86g, 88	The claims are drawn to a multivalent vaccine wherein gag and pol are expressed from two individual vectors.	
41	861, 88, 89	The claims are drawn to a multivalent vaccine wherein gag and pol are expressed individually from one vector.	
42	86i, 88	The claims are drawn to a multivalent vaccine wherein pol and nef are expressed from two individual vectors.	
43	86j, 88, 89	The claims are drawn to a multivalent vaccine wherein pol and nef are expressed from individually from one vector.	
44	86k, 88	The claims are drawn to a multivalent vaccine wherein nef and gag are expressed individually from one vector.	
45	861, 88, 89	The claims are drawn to a multivalent vaccine wherein nef and gag are expressed individually from one vector.	
46	86m, 88	The claims are drawn to a multivalent vaccine wherein gag and pol are expressed as a fusion protein from one vector.	
47	86n, 88	The claims are drawn to a multivalent vaccine wherein <i>pol</i> and <i>nef</i> are expressed as a fusion protein from one vector.	
48	860, 88	The claims are drawn to a multivalent vaccine wherein nef and gag are expressed as a fusion protein from one vector.	

The inventions listed as Groups 1-48 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The technical feature linking groups 1-33 appears to be a recombinant adenoviral vector wherein the adenoviral vector is at least partially deleted in E1 but the vector may contain more deletions, the vector contains wild type sequences including packaging signals and a gene encoding a heterologous HIV protein or fragments thereof. Erd et al. (WO 96/39178) disclose a recombinant adenoviral vector that is deleted in E1 and partially deleted in E3, the remainder of the adenoviral vector contains wild type sequences. The vector additionally contains an insertion of a heterologous protein which includes HIV proteins (see abstract and claims 1 and 5). Therefore, the technical feature linking the inventions of groups 1-45 does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art.

The special technical feature of the following groups 1-3, 7-15, 19-30 and 34-48 is considered to be the combination of sequences that is disclosed in each group, see individual claim groupings above for the different sequences. The DNA disclosed in each group is made up of a different sequence having a different structure and different function.

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The special technical feature of group 4, 16 and 31 is considered to be a method of producing recombinant adenoviral particles. Each group contains different sequences hence the resulting particles would have different structures and functions associated with the particle.

The special technical feature of group 5, 17 and 32 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors. Each group contains different sequences a encoding different protein, therefore the resulting immune response will also be different.

The special technical feature of group 6, 18 and 33 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors in conjunction with immunizing the individual a DNA plasmid vaccine. Each method contains different sequences encoding a different protein, therefore the resulting immune response will also be different.

Accordingly, groups 1-48 are not so linked by the same or corresponding technical feature as to form a single general inventive concept.

Continuation of B. FIELDS SEARCHED Item 3: WEST 2.0, STN-BIOSIS, MEDLINE adenoviral vector, deletion, HIV, Gag, polyadenylation signal, CMV promoter

Form PCT/ISA/210 (second sheet) (July 1998)

CORRECTED VERSION

(19) World Intellectual Property Organization International Bureau



(43) International Publication Date 21 March 2002 (21.03.2002)

PCT

(10) International Publication Number WO 02/022080 A3

- (51) International Patent Classification7: C12N 15/86
- (21) International Application Number: PCT/US01/28861
- (22) International Filing Date:

14 September 2001 (14.09.2001)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data: 60/233,180 15 September 2000 (15.09.2000) US

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- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA,
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- (88) Date of publication of the international search report: 2 May 2002 Date of publication of the revised international search report: 16 January 2003
- (48) Date of publication of this corrected version:

6 March 2003

(15) Information about Corrections:

see PCT Gazette No. 10/2003 of 6 March 2003, Section Π **Previous Corrections:**

see PCT Gazette No. 03/2003 of 16 January 2003, Sec-

see PCT Gazette No. 30/2002 of 25 July 2002, Section II

[Continued on next page]

(54) Title: ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS

(57) Abstract: First generation adenoviral vectors and associated recombinant adenovirus-based HIV vaccines which show enhanced stability and growth properties and greater cellular-mediated immunity are described within this specification. These adenoviral vectors are utilized to generate and produce through cell culture various adenoviral-based HIV-1 vaccines which contain HIV-1 gag, HIV-1 pol and/or HIV-1 nef polynucleotide pharmaceutical products, and biologically relevant modifications thereof. These adenovirus vaccines, when directly introduced into living vertebrate tissue, preferably a mammalian host such as a human or a non-human mammal of commercial or domestic veterinary importance, express the HIV1- Gag, Pol and/or Nef protein or biologically modification thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding HIV-1 Gag, encoding codon optimized HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNAse H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The adenoviral vaccines of the present invention, when administered alone or in a combined modality regime, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.



For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

TITLE OF THE INVENTION

ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS

5 CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit, under 35 U.S.C. §119(e), of U.S. provisional applications 60/233,180, 60/279,056, and Attorney Docket 20867PV2 (serial number unassigned), filed September 15, 2000, March 27, 2001, and September 7, 2001, respectively.

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STATEMENT REGARDING FEDERALLY-SPONSORED R&D Not Applicable

REFERENCE TO MICROFICHE APPENDIX

15 Not Applicable

FIELD OF THE INVENTION

The present invention relates to recombinant, replication-deficient first generation adenovirus vaccines found to exhibit enhanced growth properties and greater cellular-mediated immunity as compared to other replication-deficient vectors. The invention also relates to the associated first generation adenoviral vectors described herein, which, through the incorporation of additional 5' adenovirus sequence, enhance large scale production efficiency of the recombinant, replicationdefective adenovirus described herein. Another aspect of the instant invention is the surprising discovery that the intron A portion of the human cytomegalovirus (hCMV) promoter constitutes a region of instability in adenoviral vector constructs. Removal of this region from adenoviral expression constructs results in greatly improved vector stability. Therefore, improved vectors expressing a transgene under the control of an intron A-deleted CMV promoter constitute a further aspect of this invention. These adenoviral vectors are useful for generating recombinant adenovirus vaccines against human immunodeficiency virus (HIV). In particular, the first generation adenovirus vectors disclosed herein are utilized to construct and generate adenovirus-based HIV-1 vaccines which contain HTV-1 Gag, HTV-1 Pol and/or HTV-1 Nef polynucleotide pharmaceutical products, and biologically active modifications thereof. Host administration of the recombinant, replication-deficient adenovirus vaccines described herein results in expression of HIV-1 Gag, HIV-1- Pol and/or Nef protein or

immunologically relevant modifications thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding codon optimized HIV-1 Gag, HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNAse H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef, and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The HIV adenovirus vaccines of the present invention, when administered alone or in a combined modality and/or prime/boost regimen, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.

BACKGROUND OF THE INVENTION

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Human Immunodeficiency Virus-1 (HIV-1) is the etiological agent of acquired human immune deficiency syndrome (AIDS) and related disorders. HIV-1 is an RNA virus of the Retroviridae family and exhibits the 5'LTR-gag-pol-env-LTR 3' organization of all retroviruses. The integrated form of HIV-1, known as the provirus, is approximately 9.8 Kb in length. Each end of the viral genome contains flanking sequences known as long terminal repeats (LTRs). The HIV genes encode at least nine proteins and are divided into three classes; the major structural proteins (Gag, Pol, and Env), the regulatory proteins (Tat and Rev); and the accessory proteins (Vpu, Vpr, Vif and Nef).

The gag gene encodes a 55-kilodalton (kDa) precursor protein (p55) which is expressed from the unspliced viral mRNA and is proteolytically processed by the HIV protease, a product of the pol gene. The mature p55 protein products are p17 (matrix), p24 (capsid), p9 (nucleocapsid) and p6.

The pol gene encodes proteins necessary for virus replication; a reverse transcriptase, a protease, integrase and RNAse H. These viral proteins are expressed as a Gag-Pol fusion protein, a 160 kDa precursor protein which is generated via a ribosomal frame shifting. The viral encoded protease proteolytically cleaves the Pol polypeptide away from the Gag-Pol fusion and further cleaves the Pol polypeptide to the mature proteins which provide protease (Pro, P10), reverse transcriptase (RT, P50), integrase (IN, p31) and RNAse H (RNAse, p15) activities.

The *nef* gene encodes an early accessory HIV protein (Nef) which has been shown to possess several activities such as down regulating CD4 expression, disturbing T-cell activation and stimulating HIV infectivity.

The env gene encodes the viral envelope glycoprotein that is translated as a 160-kilodalton (kDa) precursor (gp160) and then cleaved by a cellular protease to yield the external 120-kDa envelope glycoprotein (gp120) and the transmembrane 41-kDa envelope glycoprotein (gp41). Gp120 and gp41 remain associated and are displayed on the viral particles and the surface of HIV-infected cells.

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The *tat* gene encodes a long form and a short form of the Tat protein, a RNA binding protein which is a transcriptional transactivator essential for HIV-1 replication.

The *rev* gene encodes the 13 kDa Rev protein, a RNA binding protein. The Rev protein binds to a region of the viral RNA termed the Rev response element (RRE). The Rev protein promotes transfer of unspliced viral RNA from the nucleus to the cytoplasm. The Rev protein is required for HIV late gene expression and in turn, HIV replication.

- Gp120 binds to the CD4/chemokine receptor present on the surface of helper T-lymphocytes, macrophages and other target cells in addition to other co-receptor molecules. X4 (macrophage tropic) virus show tropism for CD4/CXCR4 complexes while a R5 (T-cell line tropic) virus interacts with a CD4/CCR5 receptor complex. After gp120 binds to CD4, gp41 mediates the fusion event responsible for virus entry. The virus fuses with and enters the target cell, followed by reverse transcription of its single stranded RNA genome into the double-stranded DNA via a RNA dependent DNA polymerase. The viral DNA, known as provirus, enters the cell nucleus, where the viral DNA directs the production of new viral RNA within the nucleus, expression of early and late HIV viral proteins, and subsequently the production and cellular release of new virus particles. Recent advances in the ability to detect viral load within the host shows that the primary infection results in an extremely high generation and tissue distribution of the virus, followed by a steady state level of virus (albeit through a continual viral production and turnover during this phase), leading ultimately to another burst of virus load which leads to the onset of clinical AIDS. Productively infected cells have a half life of several days, whereas chronically or latently infected cells have a 3-week half life, followed by non-productively infected cells which have a long half life (over 100 days) but do not significantly contribute to day to day viral loads seen throughout the course of disease.

Destruction of CD4 helper T lymphocytes, which are critical to immune defense, is a major cause of the progressive immune dysfunction that is the hallmark of HIV infection. The loss of CD4 T-cells seriously impairs the body's ability to fight most invaders, but it has a particularly severe impact on the defenses against viruses, fungi, parasites and certain bacteria, including mycobacteria.

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Effective treatment regimens for HTV-1 infected individuals have become available recently. However, these drugs will not have a significant impact on the disease in many parts of the world and they will have a minimal impact in halting the spread of infection within the human population. As is true of many other infectious diseases, a significant epidemiologic impact on the spread of HIV-1 infection will only occur subsequent to the development and introduction of an effective vaccine. There are a number of factors that have contributed to the lack of successful vaccine development to date. As noted above, it is now apparent that in a chronically infected person there exists constant virus production in spite of the presence of anti-HIV-1 humoral and cellular immune responses and destruction of virally infected cells. As in the case of other infectious diseases, the outcome of disease is the result of a balance between the kinetics and the magnitude of the immune response and the pathogen replicative rate and accessibility to the immune response. Pre-existing immunity may be more successful with an acute infection than an evolving immune response can be with an established infection. A second factor is the considerable genetic variability of the virus. Although anti-HIV-1 antibodies exist that can neutralize HIV-1 infectivity in cell culture, these antibodies are generally virus isolate-specific in their activity. It has proven impossible to define serological groupings of HIV-1 using traditional methods. Rather, the virus seems to define a serological "continuum" so that individual neutralizing antibody responses, at best, are effective against only a handful of viral variants. Given this latter observation, it would be useful to identify immunogens and related delivery technologies that are likely to elicit anti-HTV-1 cellular immune responses. It is known that in order to generate CTL responses antigen must be synthesized within or introduced into cells, subsequently processed into small peptides by the proteasome complex, and translocated into the endoplasmic reticulum/Golgi complex secretory pathway for eventual association with major histocompatibility complex (MHC) class I proteins. CD8⁺ T lymphocytes recognize antigen in association with class I MHC via the T cell receptor (TCR) and the CD8 cell surface protein. Activation of naive CD8⁺ T cells into activated effector or memory cells generally requires both TCR engagement of antigen as described above as well as engagement of costimulatory proteins. Optimal

induction of CTL responses usually requires "help" in the form of cytokines from CD4⁺ T lymphocytes which recognize antigen associated with MHC class II molecules via TCR and CD4 engagement.

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European Patent Applications 0 638 316 (Published February 15, 1995) and 0 586 076 (Published March 9, 1994), (both assigned to American Home Products Corporation) describe replicating adenovirus vectors carrying an HIV gene, including env or gag. Various treatment regimens were used with chimpanzees and dogs, some of which included booster adenovirus or protein plus alum treatments.

Replication-defective adenoviral vectors harboring deletions in the E1 region are known, and recent adenoviral vectors have incorporated the known packaging repeats into these vectors; e.g., see EP 0 707 071, disclosing, *inter alia*, an adenoviral vector deleted of E1 sequences from base pairs 459 to 3328; and U.S. Patent No. 6,033,908, disclosing, *inter alia*, an adenoviral vector deleted of base pairs 459-3510. The packaging efficiency of adenovirus has been taught to depend on the number of incorporated individual A (packaging) repeats; *see*, *e.g.*, Gräble and Hearing, 1990 *J. Virol.* 64(5):2047-2056; Gräble and Hearing, 1992 *J. Virol.* 66(2):723-731.

Larder, et al., (1987, Nature 327: 716-717) and Larder, et al., (1989, Proc. Natl. Acad. Sci. 86: 4803-4807) disclose site specific mutagenesis of HIV-1 RT and the effect such changes have on in vitro activity and infectivity related to interaction with known inhibitors of RT.

Davies, et al. (1991, *Science* 252:, 88-95) disclose the crystal structure of the RNase H domain of HIV-1 Pol.

Schatz, et al. (1989, FEBS Lett. 257: 311-314) disclose that mutations Glu478Gln and His539Phe in a complete HIV-1 RT/RNase H DNA fragment results in defective RNase activity without effecting RT activity.

Mizrahi, et al. (1990, Nucl. Acids. Res. 18: pp. 5359-5353) disclose additional mutations Asp443Asn and Asp498Asn in the RNase region of the pol gene which also results in defective RNase activity. The authors note that the Asp498Asn mutant was difficult to characterize due to instability of this mutant protein.

Leavitt, et al. (1993, *J. Biol. Chem.* 268: 2113-2119) disclose several mutations, including a Asp64Val mutation, which show differing effect on HIV-1 integrase (IIN) activity.

Wiskerchen, et al. (1995, J. Virol. 69: 376-386) disclose singe and double mutants, including mutation of aspartic acid residues which effect HIV-1 IN and viral replication functions.

It would be of great import in the battle against AIDS to produce a prophylactic- and/or therapeutic-based HIV vaccine which generates a strong cellular immune response against an HIV infection. The present invention addresses and meets these needs by disclosing a class of adenovirus vaccines which, upon host administration, express codon optimized and modified versions of the HIV-1 genes, gag, pol and nef. These recombinant, replication-defective adenovirus vaccines may be administered to a host, such as a human, alone or as part of a combined modality regimen and/or prime-boost vaccination regimen with components of the present invention and/or a distinct viral HIV DNA vaccine, non-viral HIV DNA vaccine, HIV subunit vaccine, an HIV whole killed vaccine and/or a live attenuated HIV vaccine.

SUMMARY OF THE INVENTION

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The present invention relates to enhanced replication-defective recombinant adenovirus vaccine vectors and associated recombinant, replication-deficient adenovirus vaccines which encode various forms of HIV-1 Gag, HIV-1 Pol, and/or HIV-1 Nef, including immunologically relevant modifications of HIV-1 Gag, HIV-1 Pol and HIV-1 Nef. The adenovirus vaccines of the present invention express HIV antigens and provide for improved cellular-mediated immune responses upon host administration. Potential vaccinees include but are not limited to primates and especially humans and non-human primates, and also include any non-human mammal of commercial or domestic veterinary importance. An effect of the improved recombinant adenovirus-based vaccines of the present invention should be a lower transmission rate to previously uninfected individuals (i.e., prophylactic applications) and/or reduction in the levels of the viral loads within an infected individual (i.e., therapeutic applications), so as to prolong the asymptomatic phase of HIV-1 infection. In particular, the present invention relates to adenoviral-based vaccines which encode various forms of codon optimized HIV-1 Gag (including but in no way limited to p55 versions of codon optimized full length (FL) Gag and tPA-Gag fusion proteins), HIV-1 Pol, HIV-1 Nef, and selected modifications of immunological relevance. The administration, intracellular delivery and expression of these adenovirus vaccines elicit a host CTL and Th response. The preferred replication-defective recombinant adenoviral vaccine vectors include but are not limited to synthetic DNA molecules which (1) encode codon optimized versions of wild type HIV-1 Gag; (2) encode codon optimized versions of HIV-1 Pol; (3) encode codon optimized versions of HIV-1 Pol fusion proteins; (4) encode codon optimized versions of modified HIV-1 Pol proteins and fusion proteins, including but not limited

to pol modifications involving residues within the catalytic regions responsible for RT, RNase and IN activity within the host cell; (5) encode codon optimized versions of wild type HIV-1 Nef; (6) codon optimized versions of HIV-1 Nef fusion proteins; and/or (7) codon optimized versions of HIV-1 Nef derivatives, including but not limited to nef modifications involving introduction of an amino-terminal leader sequence, removal of an amino-terminal myristylation site and/or introduction of dileucine motif mutations. The Nef-based fusion and modified proteins, disclosed within this specification and expressed from an adenoviral-based vector vaccine this specification, may possess altered trafficking and/or host cell function while retaining the ability to be properly presented to the host MHC I complex and in turn elicit a host CTL and Th response. Examples of HIV-1 Gag, Pol and/or Nef fusion proteins include but are not limited to fusion of a leader or signal peptide at the NH₂-teriminal portion of the viral antigen coding region. Such a leader peptide includes but is not limited to a tPA leader peptide.

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The adenoviral vector utilized in construction of the HIV-1 Gag-, HIV-1 Poland/or HIV-1 Nef- based vaccines of the present invention may comprise any replication-defective adenoviral vector which provides for enhanced genetic stability of the recombinant adenoviral genome through large scale production and purification of the recombinant virus. In other words, an HIV-1 Gag-, Pol- or Nef-based adenovirus vaccine of the present invention is a purified recombinant, replicationdefective adenovirus which is shown to be genetically stable through multiple passages in cell culture and remains so during large scale production and purification procedures. Such a recombinant adenovirus vector and harvested adenovirus vaccine lends itself to large scale dose filling and subsequent worldwide distribution procedures which will be demanded of an efficacious monovalent or multivalent HIV vaccine. The present invention meets this basic requirement with description of a replication-defective adenoviral vector and vectors derived therefrom, at least partially deleted in E1, comprising a wildtype adenovirus cis-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 of the wildtype adenovirus genome. A preferred embodiment of the instant invention comprises base pairs 1-450 of a wildtype adenovirus. In other preferred embodiments, the replication -defective adenoviral vector has, in addition thereto, a region 3' to the E1-deleted region comprising base pairs 3511-3523. Basepairs 342-450 (more particularly, 400-450) constitute an extension of the 5'region of previously disclosed vectors carrying viral antigens, particularly HIV antigens (see, e.g., PCT International Application PCT/US00/18332, published

January 11, 2001 (WO 01/02067), which claims priority to U.S. Provisional Application Serial Nos. 60/142,631 and 60/148,981, filed 7/6/1999 and 8/13/1999, respectively; these documents herein incorporated by reference. Applicants have found that extending the 5' region further into the E1 gene into the disclosed vaccine vectors incorporated elements found to be important in optimizing the packaging of the virus.

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As compared to previous vectors not comprising basepairs from about 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 of the wildtype adenovirus genome, vectors comprising the above region exhibited enhanced 10 growth characteristics, with approximately 5-10 fold greater amplification rates, a more potent virus effect, allowing lower doses of virus to be used to generate equivalent immunity; and a greater cellular-mediated immune response than replication-deficient vectors not comprising this region (basepairs 1-450). Even more important, adenoviral constructs derived therefrom are very stable genetically in large-scale production, particularly those comprising an expression cassette under the 15 control of a hCMV promoter devoid of intron A. This is because Applicants have surprisingly found that the intron A portion of the hCMV promoter constituted a region of instability when employed in adenoviral vectors. Applicants have, therefore, identified an enhanced adenoviral vector which is particularly suited for use 20 in gene therapy and nucleotide-based vaccine vectors which, favorably, lends itself to large scale propagation.

A preferred embodiment of this invention is a replication-defective adenoviral vector in accordance with the above description wherein the gene is inserted in the form of a gene expression cassette comprising (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and, (c) a transcription terminator.

In preferred embodiments, the E1 gene, other than that contained within basepairs 1-450 or, alternatively, that contained within base pairs 1-450 and 3511-3523 has been deleted from the adenoviral vector, and the gene expression cassette has replaced the deleted E1 gene. In other preferred embodiments, the replication defective adenovirus genome does not have a functional E3 gene, or the E3 gene has been deleted. Most preferably, the E3 region is present within the adenoviral genome. Further preferred embodiments are wherein the gene expression cassette is in an E1 anti-parallel (transcribed in a 3' to 5' direction relative to the vector backbone)

orientation or, more preferably, an E1 parallel (transcribed in a 5' to 3' direction relative to the vector backbone) orientation.

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Further embodiments relate to a shuttle plasmid vector comprising: an adenoviral portion and a plasmid portion, wherein said adenovirus portion comprises: a) a replication defective adenovirus genome, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 (preferably, 1-450) of the wildtype adenovirus genome and, preferably, in addition thereto, basepairs 3511-3523 of a wildtype adenovirus sequence; and b) a gene expression cassette comprising: (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and (c) a transcription terminator and/or a polyadenylation site.

Other aspects of this invention include a host cell comprising said adenoviral vectors and/or said shuttle plasmid vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and (b) harvesting the resultant adenoviral vectors.

To this end, the present invention particularly relates to harvested recombinant, replication defective virus derived from a host cell, such as but not limited to 293 cells or PER.C6® cells, including but not limited to harvested virus related to any of the MRKAd5 vector backbones, with or without an accompanying transgene, including but not limited to the HIV-1 antigens described herein. An HIV-1 vaccine is represented by any harvested, recombinant adenovirus material which expresses any one or more of the HIV-1 antigens disclosed herein. This harvested material may then be purified, formulated and stored prior to host administration.

Another aspect of this invention is a method of generating a cellular immune response against a protein in an individual comprising administering to the individual an adenovirus vaccine vector comprising:

a) a recombinant, replication defective adenoviral vector, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting adenovirus packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 (preferably, 1-450) and, preferably in addition thereto, base pairs 3511-3523 of a wildtype adenovirus sequence, and,

b) a gene expression cassette comprising:(i) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (ii) a heterologous promoter operatively linked to the nucleic acid of part a); and (iii) a transcription terminator and/or a polyadenylation site.

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In view of the efficacious nature of the adenoviral and/or DNA plasmid vaccines described herein, the present invention relates to all methodology regarding administration of one or more of these adenoviral and/or DNA plasmid vaccines to provide effective immunoprophylaxis, to prevent establishment of an HIV-1 infection following exposure to this virus, or as a post-HIV infection therapeutic vaccine to mitigate the acute HIV-1 infection so as to result in the establishment of a lower virus load with beneficial long term consequences. As discussed herein, such a treatment regimen may include a monovalent or multivalent composition, various combined modality applications, and/or a prime/boost regimen to as to optimize antigen expression and a concomitant cellular-mediated and/or humoral immune response upon inoculation into a living vertebrate tissue. Therefore, the present invention provides for methods of using the adenoviral and/or DNA plasmid vaccines disclosed herein within the various parameters disclosed herein as well as any additional parameters known in the art, which, upon introduction into mammalian tissue induces intracellular expression of the gag, pol and/or nef-based vaccines.

To this end, the present invention relates in part to methods of generating a cellular immune response in a vaccinee, preferably a human vaccinee, wherein the individual is given more than one administration of adenovirus vaccine vector, and it may be given in a regimen accompanied by the administration of a plasmid vaccine. The plasmid vaccine (also referred to herein as a "DNA plasmid vaccine" or "vaccine plasmid" comprises a nucleic acid encoding a protein or an immunologically relevant portion thereof, a heterologous promoter operably linked to the nucleic acid sequence, and a transcription terminator or a polyadenylation signal (such as bGH or SPA, respectively). There may be a predetermined minimum amount of time separating the administrations. The individual can be given a first dose of plasmid vaccine, and then a second dose of plasmid vaccine. Alternatively, the individual may be given a first dose of adenovirus vaccine, and then a second dose of adenovirus vaccine. In other embodiments, the plasmid vaccine is administered first, followed after a time by administration of the adenovirus vaccine. Conversely, the adenovirus vaccine may be administered first, followed by administration of plasmid vaccine after a time. In these embodiments, an individual may be given multiple doses of the same adenovirus serotype in either viral vector or plasmid form, or the virus may be of

differing serotypes. In the alternative, a viral antigen of interest can be first delivered via a viral vaccine other than an adenovirus-based vaccine, and then followed with the adenoviral vaccine disclosed. Alternative viral vaccines include but are not limited to pox virus and venezuelan equine encephilitis virus.

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The present invention also relates to multivalent adenovirus vaccine compositions which comprise Gag, Pol and Nef components described herein; see, e.g., Example 29 and Table 25. Such compositions will provide for an enhanced cellular immune response subsequent to host administration, particularly given the genetic diversity of human MHCs and of circulating virus. Examples, but not limitations, include MRKAd5-vector based multivalent vaccine compositions which provide for a divalent (i.e., gag and nef, gag and pol, or pol and nef components) or a trivalent vaccine (i.e., gag, pol and nef components) composition. Such a mutlivalent vaccine may be filled for a single dose or may consist of multiple inoculations of each individually filled component; and may in addition be part of a prime/boost regimen with viral or non-viral vector vaccines as introduced in the previous paragraph. To this end, preferred compositions are MRKAd5 adenovirus used in combination with multiple, distinct HIV antigen classes. Each HIV antigen class is subject to sequence manipulation, thus providing for a multitude of potential vaccine combinations; and such combinations are within the scope of the present invention. The utilization of such combined modalities vaccine formulation and administration increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

The concept of a "combined modality" as disclosed herein also covers the alternative mode of administration whereby multiple HIV-1 viral antigens may be ligated into a proper shuttle plasmid for generation of a pre-adenoviral plasmid comprising multiple open reading frames. For example, a trivalent vector may comprise a gag-pol-nef fusion, in either a E3(-) or E3(+) background, preferably a E3 deleted backbone, or possibly a "2+1" divalent vaccine, such as a gag-pol fusion (i.e., codon optimized p55 gag and inactivated optimized pol; Example 29 and Table 25) within the same MRKAd5 backbone, with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES). Therefore, a multivalent vaccine delivered as a single, or possibly a second harvested recombinant, replication-deficient adenovirus is contemplated as part of the present invention.

Therefore, the adenoviral vaccines and plasmid DNA vaccines of this invention may be administered alone, or may be part of a prime and boost administration regimen. A mixed modality priming and booster inoculation scheme will result in an enhanced immune response, particularly if pre-existing anti-vector immune responses are present. This one aspect of this invention is a method of priming a subject with the plasmid vaccine by administering the plasmid vaccine at least one time, allowing a predetermined length of time to pass, and then boosting by administering the adenoviral vaccine. Multiple primings typically, 1-4, are usually employed, although more may be used. The length of time between priming and boost may typically vary from about four months to a year, but other time frames may be used. In experiments with rhesus monkeys, the animals were primed four times with plasmid vaccines, then were boosted 4 months later with the adenoviral vaccine. Their cellular immune response was notably higher than that of animals which had only received adenoviral vaccine. The use of a priming regimen may be particularly preferred in situations where a person has a pre-existing anti-adenovirus immune response.

It is an object of the present invention to provide for enhanced replication-defective recombinant adenoviral vaccine vector backbones. These recombinant adenoviral backbones may accept one or more transgenes, which may be passaged through cell culture for growth, amplification and harvest.

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It is a further object to provide for enhanced replication-defective recombinant adenoviral vaccine vectors which encode various transgenes.

It is also an object of the present invention to provide for a harvested recombinant, replication-deficient adenovirus which shows enhanced growth and amplification rates while in combination with increased virus stability after continuous passage in cell culture. Such a recombinant adenovirus is particularly suited for use in gene therapy and nucleotide-based vaccine vectors which, favorably, lends itself to large scale propagation.

To this end, it is an object of the present invention to provide for (1) enhanced replication-defective recombinant adenoviral vaccine vectors as described herein which encode various forms of HIV-1 Gag, HIV-1 Pol, and/or HIV-1 Nef, including immunologically relevant modifications of HIV-1 Gag, HIV-1 Pol and HIV-1 Nef, and (2) harvested, purified recombinant replication-deficient adenovirus generated by passage of the adenoviral vectors of (1) through one or multiple passages through cell culture, including but not limited to passage through 293 cells or PER.C6® cells.

It is also an object of the present invention to provide for recombinant adenovirus harvested by one or multiple passages through cell culture. As relating to recombinant adenoviral vaccine vector, this recombinant virus is harvested and formulated for subsequent host administration.

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It is also an object of the present invention to provide for replication-defective adenoviral vectors wherein at least one gene is inserted in the form of a gene expression cassette comprising (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and, (c) a transcription terminator.

It is also an object of the present invention to provide for a host cell comprising said adenoviral vectors and/or said shuttle plasmid vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and (b) harvesting the resultant adenoviral vectors. It is a further object of the present invention to provide for methods of generating a cellular immune response against a protein in an individual comprising administering to the individual an adenovirus vaccine vector comprising a) a replication defective adenoviral vector, at least partially deleted in E1, comprising a wildtype adenovirus cis-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about 450 (preferably, 1-450) and, preferably, 3511-3523 of a wildtype adenovirus sequence, and, b) a gene expression cassette comprising:(i) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (ii) a heterologous promoter operatively linked to the nucleic acid of part a); and (iii) a transcription terminator and/or a polyadenylation site.

It is also an object of the present invention to provide various alternatives for vaccine administration regimes, namely administration of one or more adenoviral and/or DNA plasmid vaccines described herein to provide effective immunoprophylaxis for uninfected individuals or a therapeutic treatment for HIV infected patients. Such processes include but are not limited to multivalent HIV-1 vaccine compositions, various combined modality regimes as well as various prime/boost alternatives. These methods of administration, relating to vaccine composition and/or scheduled administration, will increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

As used throughout the specification and claims, the following definitions and abbreviations are used:

"HAART" refers to - highly active antiretroviral therapy -.

"first generation" vectors are characterized as being replication-defective.

They typically have a deleted or inactivated E1 gene region, and preferably have a deleted or inactivated E3 gene region as well.

"AEX" refers to Anion Exchange chromatography.

"QPA" refers to Quick PCR-based Potency Assay.

"bps" refers to basepairs.

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"s" or "str" denotes that the transgene is in the E1 parallel or "straight" orientation.

"PBMCs" refers to peripheral blood monocyte cells.

"FL" refers to full length.

"FLgag" refers to a full-length optimized gag gene, as shown in Figure 2.

"Ad5-Flgag" refers to an adenovirus serotype 5 replication deficient virus which carries an expression cassette which comprises a full length optimized gag gene under the control of a CMV promoter.

"Promoter" means a recognition site on a DNA strand to which an RNA polymerase binds. The promoter forms an initiation complex with RNA polymerase to initiate and drive transcriptional activity. The complex can be modified by activating sequences such as enhancers or inhibiting sequences such as silencers.

"Leader" means a DNA sequence at the 5' end of a structural gene which is transcribed along with the gene. This usually results a protein having an N-terminal peptide extension, often referred to as a pro-sequences.

"Intron" means a section of DNA occurring in the middle of a gene which does not code for an amino acid in the gene product. The precursor RNA of the intron is excised and is therefore not transcribed into mRNA not translated into protein.

"Immunologically relevant" or "biologically active" means (1) with regards to a viral protein, that the protein is capable, upon administration, of eliciting a measurable immune response within an individual sufficient to retard the propagation and/or spread of the virus and/or to reduce the viral load present within the individual; or (2) with regards to a nucleotide sequence, that the sequence is capable of encoding for a protein capable of the above.

"Cassette" refers to a nucleic acid sequence which is to be expressed, along with its transcription and translational control sequences. By changing the cassette, a vector can express a different sequence.

"bGHpA" refers to the bovine growth hormone transcription terminator/polyadenylation sequence.

"tPAgag" refers to a fusion between the leader sequence of the tissue plasminogen activator leader sequence and an optimized HIV gag gene, as exemplified in Figure 30A-B, whether in a DNA or adenovirus-based vaccine vector.

Where utilized, "IA" or "inact" refers to an <u>inactivated</u> version of a gene (e.g. IApol).

"MCS" is "multiple cloning site".

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In general, adenoviral constructs, gene constructs are named by reference to the genes contained therein. For example:

"Ad5 HIV-1 gag", also referred to as the original HIV-1 gag adenoviral vector, is a vector containing a transgene cassette composed of a hCMV intron A promoter, the full length version of the human codon-optimized HIV-1 gag gene, and the bovine growth hormone polyadenylation signal. The transgene was inserted in the E1 antiparallel orientation in an E1 and E3 deleted adenovector.

"MRK Ad5 HIV-1 gag" also referred to as "MRKAd5gag" or "Ad5gag2" is an adenoviral vector taught herein which is deleted of E1, comprises basepairs 1-450 and 3511-3523, and has a human codon-optimized HIV-1 gene in an E1 parallel orientation under the control of a CMV promoter without intron A. The construct also comprises a bovine growth hormone polyadenylation signal.

"pV1JnsHIVgag", also referred to as "HIVFLgagPR9901", is a plasmid comprising the CMV immediate-early (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone.

"pV1JnsCMV(no intron)-FLgag-bGHpA" is a plasmid derived from pV1JnsHIVgag which is deleted of the intron A portion of CMV and which comprises the full length HIV gag gene. This plasmid is also referred to as "pV1JnsHIVgag-bGHpA", pV1Jns-hCMV-FL-gag-bGHpA" and "pV1JnsCMV(no intron) + FLgag + bGHpA".

"pV1JnsCMV(no intron)-FLgag-SPA" is a plasmid of the same composition as pV1JnsCMV(no intron)-FLgag-bGHpA except that the SPA termination sequence replaces that of bGHpA. This plasmid is also referred to as "pV1Jns-HIVgag-SPA" and pV1Jns-hCMV-FLgag-SPA".

"pdelE1sp1A" is a universal shuttle vector with no expression cassette (i.e., no promoter or polyA). The vector comprises wildtype adenovirus serotype 5 (Ad5) sequences from bp 1 to bp 341 and bp 3524 to bp 5798, and has a multiple cloning

site between the Ad5 sequences ending 341 bp and beginning 3524 bp. This plasmid is also referred to as the original Ad 5 shuttle vector.

"MRKpdelE1sp1A" or "MRKpdelE1(Pac/pIX/pack450)" or

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"MRKpdelE1(Pac/pIX/pack450)Cla1" is a universal shuttle vector with no expression cassette (i.e. no promoter or polyA) comprising wildtype adenovirus serotype 5 (Ad5) sequences from bp1 to bp450 and bp 3511 to bp 5798. The vector has a multiple cloning site between the Ad5 sequence ending 450 bp and beginning 3511 bp. This shuttle vector may be used to insert the CMV promoter and the bGHpA fragments in both the straight ("str". or E1 parallel) orientation or in the opposite (opp. or E1 antiparallel) orientation)

"MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.)" is still another shuttle vector which is the modified vector that contains the CMV promoter (no intronA) and the bGHpA fragments. The expression unit containing the hCMV promoter (no intron A) and the bovine growth hormone polyadenylation signal has been inserted into the shuttle vector such that insertion of the gene of choice at a unique BgIII site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1/E3+)Cla1 pre-plasmid. This shuttle vector, as shown in Figures 22 and 23, was used to insert the respective IApol and G2A,LLAA nef genes directly into.

"MRKpdelE1-CMV(no intron)-FLgag-bGHpA" is a shuttle comprising Ad5 sequences from basepairs 1-450 and 3511-5798, with an expression cassette containing human CMV without intron A, the full-length human codon-optimized HIV gag gene and bovine growth hormone polyadenylation signal. This plasmid is also referred to as "MRKpdelE1 shuttle +hCMV-FL-gag-BGHpA"

"MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA" is an adenoviral vector comprising all Ad5 sequences except those nucleotides encompassing the E1 region (from 451-3510), a human CMV promoter without intron A, a full-length human codon-optimized HIV gag gene, and a bovine growth hormone polyadenylation signal. This vector is also referred to as "MRKpAdHVE3 + hCMV-FL-gag-BGHpA", "MRKpAd5HIV-1gag", "MRKpAd5gag", "pMRKAd5gag" or "pAd5gag2".

"pV1Jns-HIV-pol inact(opt)" or "pV1Jns-HIV IA pol (opt) is the inactivated Pol gene (contained within SEQ ID NO:3) cloned into the BgIII site of V1Jns (Figure 17A-C). As noted herein, various derivatives of HIV-1 pol may be cloned into a plasmid expression vector such as V1Jns or V1Jns-tPA, thus serving directly as DNA vaccine candidates or as a source for subcloning into an appropriate adenoviral vector.

"MRKpdel+hCMVmin+FL-pol+bGHpA(s)" is the "MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.)" shuttle mentioned above which contains the IA pol gene is the proper orientation. This shuttle vector is used in a bacterial recombination with MRKpAd(E1-/E3+)Cla1.

"MRKpAd+hCMVmin+FL-pol+bGHpA(S)E3+", also referred to herein as "pMRKAd5pol", is the pre-adenovirus plasmid which comprises a CMV-pol inact(opt)-pGHpA construct. The construction of this pre-adenovirus plasmid is shown in Figure 22.

"pV1Jns/nef (G2A,LLAA)" or "V1Jns/opt nef (G2A,LLAA)" comprises codon optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175 (SEQ ID NO:13; which comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662). This fragment is subcloned into the Bgl II site of V1Jns and/orV1Jns-tPA (Figures 16A-B). As noted above for HIV-1 pol, HIV-1 nef constructs may be cloned into a plasmid expression vector such as V1Jns or V1Jns-tPA, thus serving directly as DNA vaccine candidates or as a source for subcloning into an appropriate adenoviral vector.

"MRKpdelE1hCMVminFL-nefBGHpA(s)", also referred to herein as "pMRKAd5nef", is the pre-adenovirus plasmid which comprises a CMV-nef (G2A,LLAA) codon optimized sequence. The construction of this pre-adenovirus plasmid is shown in Figure 23.

BRIEF DESCRIPTION OF THE FIGURES

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Figure 1 shows the original HIV-1 gag adenovector (Ad5HIV-1gag). This vector is disclosed in PCT International Application No. PCT/US00/18332 (WO 01/02607) filed July 3, 2000, claiming priority to U.S. Provisional Application Serial No. 60/142,631, filed July 6, 1999 and U.S. Application Serial No. 60/148,981, filed August 13, 1999, all three applications which are hereby incorporated by reference.

Figure 2 shows the nucleic acid sequence (SEQ ID NO: 29) of the optimized human HIV-1 gag open reading frame.

Figure 3 shows diagrammatically the new transgene constructs in comparison with the original gag transgene.

Figure 4 shows the modifications made to the original adenovector backbone in the generation of the novel vectors of the instant invention.

Figure 5 shows the virus mixing experiments that were carried out to determine the effects of the addition made to the packaging signal region (Expt. #1) and the E3 gene on viral growth (Expt. #2). The bars denote the region of modifications made to the E1 deletion.

Figure 6 shows an autoradiograph of viral DNA analysis following the viral mixing experiments described in Examples 6 and 7.

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Figures 7A, 7B and 7C are as follows: Figure 7A shows the hCMV-Flgag-bGHpA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Both E1 parallel and E1 antiparallel transgene orientation are represented. Figure 7B shows the hCMV-Flgag-SPA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Again, both E1 parallel and E1 antiparallel transgene orientation are represented. Figure 7C shows the mCMV-Flgag-bGHpA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Once again, both E1 parallel and E1 antiparallel transgene orientation are represented.

Figure 8A shows the experiment designed to test the effect of transgene orientation.

Figure 8B shows the experiments designed to test the effect of polyadenylation signal.

Figure 9 shows viral DNA from the four adenoviral vectors tested (Example 12) at P5, following *Bst*E11 digestion.

Figure 10 shows viral DNA analysis of passages 11 and 12 of MRKpAdHVE3, MRKAd5HIV-1gag, and MRKAd5HIV-1gagE3-.

Figure 11 shows viral DNA analysis (*Hind*III digestion) of passage 6 MRKpAdHVE3 and MRKAd5HIV-1gag used to initiate the viral competition study. The last two lanes are passage 11 analysis of duplicate passages of the competition study (each virus at MOI of 280 viral particles).

Figure 12 shows viral DNA analysis by *Hind* III digestion on high passage numbers for MRKAd5HIV-1gag in serum-containing media with collections made at specified times. The first lane shows the 1kb DNA size marker. The other lanes represent pre-plasmid control (digested with Pac1 and *Hind*III), MRKAd5HIV-1gag at P16, P19, and P21.

Figure 13 shows serum anti-p24 levels at 3 wks post i.m. immunization of balb/c mice (n=10) with varying doses of several Adgag constructs: (A) MRK Ad5 HIV-1 gag (through passage 5); (B) MRKAd5 hCMV-FLgag-bGHpA (E3-); (C) MRKAd5 hCMV-FLgag-SPA (E3+); (D) MRKAd5 mCMV-FLgag-bGHpA (E3+);

(E) research lot (293 cell-derived) of Ad5HIV-1 gag; and (F) clinical lot (Ad5gagFN0001) of Ad5HIV-1 gag. Reported are the geometric mean titers (GMT) for each cohort along with the standard error bars.

Figure 14 shows a restriction map of the pMRKAd5HIV-1gag vector.

Figures 15A-X illustrates the nucleotide sequence of the pMRKAd5HIV-1gag vector (SEQ ID NO:27.[coding] and SEQ ID NO:28 [non-coding]).

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Figures 16A-B shows a schematic representation of DNA vaccine expression vectors V1Jns (A) and V1Jns-tPA (B), which are utilized for HIV-1 gag, pol and nef constructs in various DNA/viral vector combined modality regimens as disclosed herein.

Figures 17A-C shows the nucleotide (SEQ ID NO:3) and amino acid sequence (SEQ ID NO:4) of IA-Pol. Underlined codons and amino acids denote mutations, as listed in Table 1.

Figure 18 shows codon optimized nucleotide and amino acid sequences through the fusion junction of tPA-pol inact(opt) (contained within SEQ ID NOs: 7 and 8, respectively). The underlined portion represents the NH₂-terminal region of IA-Pol.

Figures 19A-B show a nucleotide sequence comparison between wild type nef(jrfl) and codon optimized nef. The wild type nef gene from the jrfl isolate consists of 648 nucleotides capable of encoding a 216 amino acid polypeptide. WT, wild type sequence (SEQ ID NO:19); opt, codon-optimized sequence (contained within SEQ ID NO:1). The Nef amino acid sequence is shown in one-letter code (SEQ ID NO:2).

Figures 20A-C show nucleotide sequences at junctions between nef coding sequence and plasmid backbone of nef expression vectors V1Jns/nef (Figure 20A), V1Jns/nef(G2A,LLAA) (Figure 20B), V1Jns/tpanef (Figure 20C) and V1Jns/tpanef(LLAA) (Figure 20C, also). 5' and 3' flanking sequences of codon optimized nef or codon optimized nef mutant genes are indicated by bold/italic letters; nef and nef mutant coding sequences are indicated by plain letters. Also indicated (as underlined) are the restriction endonuclease sites involved in construction of respective nef expression vectors. V1Jns/tpanef and V1Jns/tpanef(LLAA) have identical sequences at the junctions.

Figure 21 shows a schematic presentation of nef and nef derivatives. Amino acid residues involved in Nef derivatives are presented. Glycine 2 and Leucine 174 and 175 are the sites involved in myristylation and dileucine motif, respectively. For both versions of the tpanef fusion genes, the putative leader peptide cleavage sites are

indicated with "*", and a exogenous serine residue introduced during the construction of the mutants is underlined.

Figure 22 shows diagrammatically the construction of the pre-adenovirus plasmid construct, MRKAd5Pol.

Figure 23 shows diagrammatically the construction of the pre-adenovirus plasmid construct, MRKAd5Nef.

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Figure 24 shows a comparison of clade B vs. clade C anti-gag T cell responses in clade B HIV-infected subjects.

Figure 25 shows a comparison of clade B vs. clade C anti-nef T cell responses in clade B HIV-infected subjects.

Figures 26A-AO illustrates the nucleotide sequence of the pMRKAd5HIV-1pol adenoviral vector (SEQ ID NO:32 [coding] and SEQ ID NO:33 [non-coding]), comprising the coding region of the inactivated pol gene (SEQ ID NO3).

Figures 27A-AM illustrates the nucleotide sequence of the pMRKAd5HIV-1 nef adenoviral vector (SEQ ID NO:34 [coding] and SEQ ID NO:35 [non-coding]), comprising the coding region of the inactivated pol gene (SEQ ID NO13).

Figure 28 shows the stability of MRKAd5 vectors comprising various promoter fragments (hCMV or mCMV) and terminations signals (bGH or SPA) in E3(+) or E3(-) backbones.

Figures 29A and B shows the anion-exchange HPLC viral particle concentrations of the freeze-thaw recovered cell associated virus at the 24, 36, 48, and 60 hpi time points (Figure 29A) and the timcourse QPA supernatant titers (Figure 29B) for MRKAd5gag, MRKAd5pol and MRKAd5nef.

Figure 30 shows the nucleotide sequence (SEQ ID NO:36) and amino acid sequence (SEQ ID NO:37) comprising the open reading frame of a representative tPA-gag fusion for use in the DNA and/or adenoviral vaccine disclosed herein.

Figure 31 shows the intracellular γIFN staining of PBMCs collected at week 10 (post DNA prime) and week 30 (post Ad boost). The cells were stimulated overnight in the presence or absence of the gag peptide pool. They were subsequently stained using fluorescence-tagged anti-CD3, anti-CD8, anti-CD4, and anti-γIFN monoclonal antibodies. Each plot shows all CD3+ T cells which were segregated in terms of positive staining for surface CD8 and γIFN production. The numbers in the upper right and lower right quadrants of each plot are the percentages of CD3+ cells that were CD8+γIFN+ and CD4+γIFN+, respectively.

Figure 32 shows a comparison of single-modality adenovirus immunization with DNA + adjuvant prime/adenovirus boost immunization.

Figures 33A-B show the nucleotide sequence (SEQ ID NO: 38) of the open reading frame for the gag-IApol fusion of Example 29.

Figures 34A-B show the protein sequence (SEQ ID NO:39) of the gag-IApol fustion frame.

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DETAILED DESCRIPTION OF THE INVENTION

A novel replication-defective, or "first generation," adenoviral vector suitable for use in gene therapy or nucleotide-based vaccine vectors is described. This vector is at least partially deleted in E1 and comprises a wildtype adenovirus cis-acting packaging region from about base pair 1 to between about base pair 342 (more preferably, 400) to about 458 (preferably, 1-450) and, preferably, 3511-3523 of a wild-type adenovirus sequence. It has been found that a vector of this description possesses enhanced growth characteristics, with approximately 5-10 fold greater amplification rates, and is more potent allowing lower doses of virus to be used to generate equivalent immunity. The vector, furthermore, generates a harvested recombinant adenovirus which shows greater cellular-mediated immune responses than replication-deficient vectors not comprising this region (basepairs 342-450). Adenoviral constructs derived from these vectors are, further, very stable genetically, particularly those comprising a transgene under the control of a hCMV promoter devoid of intron A. Viruses in accordance with this description were passaged continually and analyzed; see Example 12. Each virus analyzed maintained it correct genetic structure. Analysis was also carried out under propagation conditions similar to that performed in large scale production. Again, the vectors were found to possess enhanced genetic stability; see Figure 12. Following 21 passages, the viral DNA showed no evidence of rearrangement, and was highly reproducible from one production lot to the next. The outcome of all relevant tests indicate that the adenoviral vector is extremely well suited for large-scale production of recombinant, replication-deficient adenovirus, as shown herein with the data associated with Figure 28.

A preferred adenoviral vector in accordance with this description is a vector comprising basepairs 1-450, which is deleted in E3. This vector can accommodate up to approximately 7,500 base pairs of foreign DNA inserts (or exogenous genetic material). Another preferred vector is one retaining E3 which comprises basepairs 1-450. A preferred vector of this description is an E3+ vector comprising basepairs 1-450 and 3511-3523. This vector, when deleted of the region spanning basepairs 451-3510, can accommodate up to approximately, 4,850 base pairs of foreign DNA inserts

(or exogenous genetic material). The cloning capacities of the above vectors have been determined using 105% of the wildtype Ad5 sequence as the upper genome size limit.

Wildtype adenovirus serotype 5 is used as the basis for the specific basepair numbers provided throughout the specification. The wildtype adenovirus serotype 5 sequence is known and described in the art; see, Chroboczek et al., 1992 J. Virology 186:280, which is hereby incorporated by reference. Accordingly, a particular embodiment of the instant invention is a vector based on the adenovirus serotype 5 sequence. One of skill in the art can readily identify the above regions in other adenovirus serotypes (e.g., serotypes 2, 4, 6, 12, 16, 17, 24, 31, 33, and 42), regions defined by basepairs corresponding to the above basepair positions given for adenovirus serotype 5. Accordingly, the instant invention encompasses all adenoviral vectors partially deleted in E1 comprising basepairs corresponding to 1-450 (particularly, 342-450) and, preferably, 3511-3523 of a wild-type adenovirus serotype 5 (Ad5) nucleic acid sequence. Particularly preferred embodiments of the instant invention are those derived from adenoviruses like Ad5 which are classified in subgroup C (e.g., Ad2).

Vectors in accordance with the instant invention are at least partially deleted in E1. Preferably the E1 region is completely deleted or inactivated. Most preferably, the region deleted of E1 is within basepairs 451-3510. It is to be noted that the extended 5' and 3' regions of the disclosed vectors are believed to effectively reduce the size of the E1 deletion of previous constructs without overlapping any part of the E1A/E1B gene present in the cell line used, i.e., the PER.C6® cell line transfected with base pairs 459-3510. Overlap of adenoviral sequences is avoided because of the possibility of recombination. One of ordinary skill in the art can certainly appreciate that the instant invention can, therefore, be modified if a different cell line transfected with a different segment of adenovirus DNA is utilized. For purposes of exemplification, a 5' region of base pairs 1 to up to 449 is more appropriate if a cell line is transfected with adenoviral sequence from base pairs 450-3510. This holds true as well in the consideration of segments 3' to the E1 deletion.

Preferred embodiments of the instant invention possess an intact E3 region (i.e., an E3 gene capable of encoding a functional E3). Alternate embodiments have a partially deleted E3, an inactivated E3 region, or a sequence completely deleted of E3. Applicants have found, in accordance with the instant invention, that virus comprising the E3 gene were able to amplify more rapidly compared with virus not comprising an E3 gene; see Figure 6 wherein a diagnostic CsCl band corresponding to the E3+ virus

tested (5,665 bp) was present in greater amount compared with the diagnostic band of 3,010 bp corresponding to the E3- virus. These results were obtained following a virus competition study involving mixing equal MOI ratio (1:1) of adenovectors both comprising the E3 gene and not comprising the E3 gene. This increased amplification capacity of the E3+ adenovectors was subsequently confirmed with growth studies; see Table 4A, wherein the E3+ virus exhibit amplification ratios of 470, 420 and 320 as compared with the 115 and 40-50 of the E3- constructs.

As stated above, vectors in accordance with the instant invention can accommodate up to approximately 4,850 base pairs of exogenous genetic material for an E3+ vector and approximately 7,500 base pairs for an E3- vector. Preferably, the insert brings the adenoviral vector as close as possible to a wild-type genomic size (e.g., for Ad5, 35,935 basepairs). It is well known that adenovirus amplifies best when they are close to their wild-type genomic size.

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The genetic material can be inserted in an E1-parallel or an E1 anti-parallel orientation, as such is illustrated in Figure 7A, 7B, 7C and Figure 8A. Particularly preferred embodiments of the instant invention, have the insert in an E1-parallel orientation. Applicants have found, via competition experiments with plasmids containing transgenes in differing orientation (Figure 8A), that vector constructs with the foreign DNA insert in an E1-parallel orientation amplify better and actually outcompete E1-antiparallel-oriented transgenes. Viral DNA analysis of the mixtures at passage 3 and certainly at passage 6, showed a greater ratio of the virus carrying the transgene in the E1 parallel orientation as compared with the E1 anti-parallel version. By passage 10, the only viral species observed was the adenovector with the transgene in the E1 parallel orientation for both transgenes tested.

Adenoviral vectors in accordance with the instant invention are particularly well suited to effectuate expression of desired proteins, one example of which is an HIV protein, particularly an HIV full length gag protein. Exogenous genetic material encoding a protein of interest can exist in the form of an expression cassette. A gene expression cassette preferably comprises (a) a nucleic acid encoding a protein of interest; (b) a heterologous promoter operatively linked to the nucleic acid encoding the protein; and (c) a transcription terminator.

The transcriptional promoter is preferably recognized by an eukaryotic RNA polymerase. In a preferred embodiment, the promoter is a "strong" or "efficient" promoter. An example of a strong promoter is the immediate early human cytomegalovirus promoter (Chapman et al, 1991 *Nucl. Acids Res*19:3979-3986, which is incorporated by reference), preferably without intronic sequences. Most preferred

for use within the instant adenoviral vector is a human CMV promoter without intronic sequences, like intron A. Applicants have found that intron A, a portion of the human cytomegalovirus promoter (hCMV), constitutes a region of instability for adenoviral vectors. CMV without intron A has been found to effectuate (Examples 1-3) comparable expression capabilities in vitro when driving HIV gag expression and, furthermore, behaved equivalently to intron A-containing constructs in Balb/c mice in vivo with respect to their antibody and T-cell responses at both dosages of plasmid DNA tested (20 µg and 200 µg). Those skilled in the art will appreciate that any of a number of other known promoters, such as the strong immunoglobulin, or other eukaryotic gene promoters may also be used, including the EF1 alpha promoter, the murine CMV promoter, Rous sarcoma virus (RSV) promoter, SV40 early/late promoters and the beta-actin promoter.

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In preferred embodiments, the promoter may also comprise a regulatable sequence such as the Tet operator sequence. This would be extremely useful, for example, in cases where the gene products are effecting a result other than that desired and repression is sought.

The combination of the CMV promoter (devoid of the intron A region) with the BGH terminator is particularly preferred although other promoter/terminator combinations in the context of FG adenovirus may also be used.

Other embodiments incorporate a leader or signal peptide into the transgene. A preferred leader is that from the tissue-specific plasminogen activator protein, tPA. Examples include but are not limited to the various tPA-gag, tPA-pol and tPA-nef adenovirus-based vaccines disclosed throughout this specification.

In view of the improved adenovirus vectors described herein, an essential portion of the present invention are adenoviral-based HIV vaccines comprising said adenovirus backbones which may be administered to a mammalian host, preferably a human host, in either a prophylactic or therapeutic setting. The HIV vaccines of the present invention, whether administered alone or in combination regimens with other viral- or non-viral-based DNA vaccines, should elicit potent and broad cellular immune responses against HIV that will either lessen the likelihood of persistent virus infection and/or lead to the establishment of a clinically significant lowered virus load

subject to HIV infection or in combination with HAART therapy, mitigate the effects of previously established HIV infection (antiviral immunotherapy(ARI)). While any HIV antigen (e.g., gag, pol, nef, gp160, gp41, gp120, tat, rev, etc.) may be utilized in the herein described recombinant adenoviral vectors, preferred embodiments include the codon optimized p55 gag antigen (herein exemplified as MRKAd5gag), pol and nef. Sequences based on different Clades of HIV-1 are suitable for use in the instant invention, most preferred of which are Clade B and Clade C. Particularly preferred embodiments are those sequences (especially, codon-optimized sequences) based on concensus Clade B sequences. Preferred versions of the MRKAd5pol and MRKAd5nef series of adenoviral vaccines will encode modified versions of pol or nef, as discussed herein. Preferred embodiments of the MRKAd5HIV-1 vectors carrying HIV envelope genes and modifications thereof comprise the HIV codon-optimized env sequences of PCT International Applications PCT/US97/02294 and PCT/US97/10517, published August 28, 1997 (WO 97/31115) and December 24, 1997, respectively; both documents of which are hereby incorporated by reference.

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A most preferred aspect of the instant invention is the disclosed use of the adenoviral vector described above to effectuate expression of HIV gag. Sequences for many genes of many HIV strains are publicly available in GENBANK and primary, field isolates of HIV are available from the National Institute of Allergy and Infectious Diseases (NIAID) which has contracted with Quality Biological (Gaithersburg, MD) to make these strains available. Strains are also available from the World Health Organization (WHO), Geneva Switzerland. It is preferred that the gag gene be from an HIV-1 strain (CAM-1; Myers et al, eds. "Human Retroviruses and AIDS: 1995, IIA3-IIA19, which is hereby incorporated by reference). This gene closely resembles the consensus amino acid sequence for the clade B (North American/European) sequence. Therefore, it is within the purview of the skilled artisan to choose an appropriate nucleotide sequence which encodes a specific HIV gag antigen, or immunologically relevant portion thereof. As shown in Example 25, a clade B or clade C based p55 gag antigen will potentially be useful on a global scale. As noted herein, the transgene of choice for insertion in to a DNA or MRKAd-based adenoviral vector of the present invention is a codon optimized version of p55 gag. Such a MRKAd5gag adenoviral vector is documented in Example 11 and is at least referred to herein as MRKAd5HIV-1gag. Of course, additional versions are contemplated, including but not limited to modifications such as promoter (e.g., mCMV for hCMV) and/or pA-terminations signal (SPA for bGH) switching, as well as generating MRK Ad5 backbones with or without deletion of the Ad5 E3 gene.

The present invention also relates a series of MRKAd5pol-based adenoviral vaccines which are shown herein to generate cellular immune responses subsequent to administration in mice and non-human primate studies. Several of the MRKAd5pol series are exemplified herein. One such adenoviral vector is referred to as MRKAd5hCMV-inact opt pol(E3+), which comprises the MRKAd5 backbone, the hCMV promoter (no intron A), an inactivated pol transgene, and contains the Ad5 E3 gene in the adenoviral backbone. A second exemplified pre-adenovirus plasmid and concomitant virus is referred to as MRKAd5hCMV-inact opt pol(E3-), which is identical to the former adenoviral vector except that the E3 is deleted. Both constructions contain a codon optimized, inactivated version of HIV-1 Pol, wherein at least the entire coding region is disclosed herein as SEQ ID NO:3 and the expressed protein is shown as SEQ ID NO:4 (see also Figure 17A-C and Table 1, which show targeted deletion for inactivated pol. This and other preferred codon optimized versions of HTV Pol as disclosed herein are essentially as described in U.S. Application Serial No. 09/745,221, filed December 21, 2000 and PCT International 15 Application PCT/US00/34724, also filed December 21, 2000, both documents which are hereby incorporated by reference. As disclosed in the above-mentioned documents, the open reading frame for these codon-optimized HIV-1 Pol-based DNA vaccines are represented by codon optimized DNA molecules encoding codon optimized HIV-1 Pol (e.g. SEQ ID NO:2), codon optimized HIV-1 Pol fused to an 20 amino terminal localized leader sequence (e.g. SEQ ID NO:6), and especially preferable, and exemplified by the MRKAd5-Pol construct in e.g., Example 19, biologically inactivated pol ("inact opt Pol"; e.g., SEQ ID NO:4) which is devoid of significant PR, RT, RNase or IN activity associated with wild type Pol. In addition, a construct related to SEO ID NO:4 is contemplated which contains a leader peptide at 25 the amino terminal region of the IA Pol protein. A specific construct is ligated within an appropriate DNA plasmid vector containing regulatory regions operatively linked to the respective HIV-1 Pol coding region, with or without a nucleotide sequence encoding a functional leader peptide. To this end, various HIV-1 Pol constructs disclosed herein relate to open reading frames for cloning to the enhanced first generation Ad vectors of the present invention (such a series of MRKAd5pol adenoviral vaccine vectors), including but not limited to wild type Pol (comprising the DNA molecule encoding WT opt Pol, as set forth in SEQ ID NO:2), tPA-opt WTPol, (comprising the DNA molecule encoding tPA Pol, as set forth in SEQ ID NO:6), inact 35 opt Pol (comprising the DNA molecule encoding IA Pol, as set forth in SEQ ID NO:4), and tPA-inact opt Pol, (comprising the DNA molecule encoding tPA-inact opt

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Pol, as set forth in SEQ ID NO:8). The pol-based versions of enhanced first generation adenovirus vaccines elicit CTL and Th cellular immune responses upon administration to the host, including primates and especially humans. As noted in the above, an effect of the cellular immune-directed vaccines of the present invention should be a lower transmission rate to previously uninfected individuals and/or reduction in the levels of the viral loads within an infected individual, so as to prolong the asymptomatic phase of HIV-1 infection.

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The present invention further relates to a series of MRKAd5nef-based adenoviral vaccines which, similar to HIV gag and pol antigens, generate cellular immune responses subsequent to administration in mice and non-human primate studies. The MRKAd5nef series are exemplified herein by utilizing the improved MRK adenoviral backbone in combination with modified versions of HIV nef. These exemplified MRKAd5nef vectors are as follows: (1) MRKAd5hCMVnef(G2A,LLAA) (E3+), which comprises the improved MRKAd5 backbone, a human CMV promoter an intact Ad5 E3 gene and a modified nef gene: (2) MRKAd5mCMVnef(G2A,LLAA) (E3+), which is the same as (1) above but substituting a murine CMV promoter for a human CMV promoter; and (3) MRKAd5mCMV-tpanef(LLAA) (E3+), which is the same as (2) except that the nef transgene is tpanef(LLAA). Codon optimized versions of HIV-1 Nef and HIV-1 Nef modifications are essentially as described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference. Particular embodiments of codon optimized Nef and Nef modifications relate to a DNA molecule encoding HIV-1 Nef from the HTV-1 ifrl isolate wherein the codons are optimized for expression in a mammalian system such as a human. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:9, while the expressed open reading frame is disclosed herein as SEQ ID NO:10. Another embodiment of Nef-based coding regions for use in the adenoviral vectors of the present invention comprise a codon optimized DNA molecule encoding a protein containing the human plasminogen activator (tpa) leader peptide fused with the NH2-terminus of the HIV-1 Nef polypeptide. The DNA molecule which encodes this protein is disclosed herein as SEO ID NO:11, while the expressed open reading frame is disclosed herein as SEQ ID NO:12. Another modified Nef optimized coding region relates to a DNA molecule encoding optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175, herein

described as opt nef (G2A, LLAA). The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:13, while the expressed open reading frame is disclosed herein as SEQ ID NO:14. MRKAd5nef vectors (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+) and (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+) contain this transgene. An additional embodiment relates to a DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA), comprises an open reading frame which encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175, herein referred to as opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, while the expressed open reading frame is disclosed herein as SEQ ID NO:16. The MRKAd5nef vector "MRKAd5mCMV-tpanef(LLAA) (E3+)" contains this transgene.

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Along with the improved MRKAd5gag adenovirus vaccine vector described herein, generation of a MRKAd5pol and MRKAd5nef adenovirus vector provide for enhanced HIV vaccine capabilities. Namely, the generation of this trio of adenoviral vaccine vectors, all shown to generate effective cellular immune responses subsequent to host administration, provide for the ability to administer these vaccine candidates not only alone, but preferably as part of a divalent (i.e., gag and nef, gag and pol, or pol and nef components) or a trivalent vaccine (i.e., gag, pol and nef components). Therefore, a preferred aspect of the present invention are vaccine formulations and associated methods of administration and concomitant generation of host cellular immune responses associated with formulating three separate series of MRKAd5based adenoviral vector vaccines. Of course, this MRKAd5 vaccine series based on distinct HIV antigens promotes expanded opportunities for formulation of a divalent or trivalent vaccine, or possibly administration of separate formulations of one or more monovalent or divalent formulations within a reasonable window of time. It is also within the scope of the present invention to embark on combined modality regimes which include multiple but distinct components from a specific antigen. An example, but certainly not a limitation, would be separate MRKAd5pol vectors, with one vaccine vector expressing wild type Pol (SEQ ID NO:2) and another MRKAd5pol vector expressing inactivated Pol (SEQ ID NO:6). Another example might be separate MRKAd5nef vectors, with one vaccine vector expressing the tPA/LLAA version of Nef (SEQ ID NO:16) and another MRKAd5nef vector expressing the G2A,LLAA modified version of Nef (SEQ ID NO:14). Therefore, the MRKAd5 adenoviral vectors of the present invention may be used in combination

with multiple, distinct HIV antigen classes. Each HIV antigen class is subject to sequence manipulation, thus providing for a multitude of potential vaccine combinations; and such combinations are within the scope of the present invention. The utilization of such combined modalities vaccine formulation and administration increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

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The present invention also relates to application of a mono-, dual-, or trimodality administration regime of the MRKAd5gag, pol and nef adenoviral vaccine
series in a prime/boost vaccination schedule. This prime/boost schedule may include
any reasonable combination of the MRKAd5gag, pol and nef adenoviral vaccine
series disclosed herein. In addition, a prime/boost regime may also involve other viral
and/or non-viral DNA vaccines. A preferable addition to an adenoviral vaccine
vector regime includes but is not limited to plasmid DNA vaccines, especially DNA
plasmid vaccines that contain at least one of the codon optimized gag, pol and nef
constructions, as disclosed herein.

Therefore, one aspect of this invention is the administration of the adenoviral vector containing the optimized gag gene in a prime/boost regiment in conjunction with a plasmid DNA encoding gag. To distinguish this plasmid from the adenoviralcontaining shuttle plasmids used in the construction of an adenovirus vector, this plasmid will be referred to as a "vaccine plasmid" or "DNA plasmid vaccine". Preferred vaccine plasmids for use in this administration protocol are disclosed in pending U.S. patent application 09/017,981, filed February 3, 1998 and WO98/34640, published August 13, 1998, both of which are hereby incorporated by reference. Briefly, the preferred vaccine plasmid is designated V1Jns-FLgag, which expresses the same codon-optimized gag gene as the adenoviral vectors of this invention (see Figure 2 for the nucleotide sequence of the exemplified optimized codon version of full length p55 gag). The vaccine plasmid backbone, designated V1Jns contains the CMV immediate-early (IE) promoter and intron A, a bovine growth hormone-derived polyadenylation and transcription termination sequence as the gene expression regulatory elements, and a minimal pUC backbone; see Montgomery et al., 1993, DNA Cell Biol. 12:777-783. The pUC sequence permits high levels of plasmid production in E. coli and has a neomycin resistance gene in place of an ampicillin resistance gene to provide selected growth in the presence of kanamycin. Alternatively, a vaccine plasmid which has the CMV promoter deleted of intron A can be used. Those of skill in the art will recognize that alternative vaccine plasmid

vectors may be easily substituted for these specific constructs, and this invention specifically envisions use of such alternative plasmid DNA vaccine vectors.

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Another aspect of the present invention is a prime/boost regimen which includes a vaccine plasmid which encodes an HIV pol antigen, preferably a codon optimized form of pol and also preferably a vaccine plasmid which comprises a nucleotide sequence which encodes a Pol antigen selected from the group of Pol antigens as shown in SEQ ID NOs: 2, 4, 6 and 8. The variety of potential DNA plasmid vaccines which encode various biologically active forms of HIV-1 Pol, wherein administration, intracellular delivery and expression of the HIV-1 Pol gene of interest elicits a host CTL and Th response. The preferred synthetic DNA molecules of the present invention encode codon optimized wild type Pol (without Pro activity) and various codon optimized inactivated HIV-1 Pol proteins. The HIV-1 pol open reading disclosed herein are especially preferred for pharmaceutical uses, especially for human administration as delivered via a recombinant adenoviral vaccine, especially an enhanced first generation recombinant adenoviral vaccine as described herein. Several embodiments of this portion of the invention are provided in detail below, namely DNA molecules which comprise a HIV-1 pol open reading frame, whether encoding full length pol or a modification or fusion as described herein, wherein the codon usage has been optimized for expression in a mammal, especially a human. Again, these DNA sequences are positioned appropriately within a recombinant adenoviral vector, such as the exemplified recombinant adenoviral vector described herein, so as to promote expression of the respective HIV-1 Pol gene of interest, and subsequent to administration, elicit a host CTL and Th response. Again, these preferred, but in no way limiting, pol genes are as disclosed herein and essentially as described in U.S. Application Serial No. 09/745,221, filed December 21, 2000 and PCT International Application PCT/US00/34724, also filed December 21, 2000, both documents which are hereby incorporated by reference.

A third series of vaccine plasmids which are useful in a combined modality and/or prime/boost regimen are vaccine plasmids which encode an HIV nef antigen or biologically and/or immunologically relevant modification thereof. As noted elsewhere, preferred vaccine plasmids contain a codon optimized form of nef and also preferably comprise a nucleotide sequence which encodes a Nef antigen selected from the group of Nef antigens as shown in SEQ ID NOs: 10, 12, 14 and 16. These preferred nef coding regions are disclosed herein, as well as being described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International

Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference.

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Therefore, the adenoviral vaccines and plasmid DNA vaccines of this invention may be administered alone, or may be part of a prime and boost administration regimen. A mixed modality priming and booster inoculation scheme will result in an enhanced immune response, particularly is pre-existing anti-vector immune responses are present. This one aspect of this invention is a method of priming a subject with the plasmid vaccine by administering the plasmid vaccine at least one time, allowing a predetermined length of time to pass, and then boosting by administering the adenoviral vaccine. Multiple primings typically, 1-4, are usually employed, although more may be used. The length of time between priming and boost may typically vary from about four months to a year, but other time frames may be used. In experiments with thesus monkeys, the animals were primed four times with plasmid vaccines, then were boosted 4 months later with the adenoviral vaccine. Their cellular immune response was notably higher than that of animals which had only received adenoviral vaccine. The use of a priming regimen may be particularly preferred in situations where a person has a pre-existing anti-adenovirus immune response.

Furthermore and in the alternative, multiple HIV-1 viral antigens, such as the MRKAd5 adenoviral vaccines disclosed herein, may be ligated into a proper shuttle plasmid for generation of a pre-adenoviral plasmid comprising multiple open reading frames. For example a trivalent vector may comprise a gag-pol-nef fusion, in either a E3(-) or E3(+) background, preferably a E3 deleted backbone, or possible a "2+1" divalent vaccine, such as a gag-pol fusion (i.e., codon optimized p55 gag and inactivated optimized pol; Example 29 and Table 25) within the same MRKAd5 backbone, with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES), as disclosed in International Publication No. WO 95/24485, which is hereby incorporated by reference. Figure 9 shows that the use of multiple promoters and termination sequences provide for similar growth properties, while Figure 28 shows that these MRKAd5gag-based vectors are also stable at least through passage 21. In the absence of the use of IRES-based technology, it is preferred that a distinct promoter be used to support each respective open reading frame, so as to best preserve vector stability. As examples, and certainly not as limitations, potential multiple transgene vaccines may

include a three transgene vector such as hCMV-gagpol-bGHpA + mCMV-nef-SPA in an E3 deleted backbone or hCMV-gagpol-bGHpA + mCMV-nef-SPA(E3+). Potential "2+1" divalent vaccines of the present invention might be a hCMV-gagbGHpA + mCMV-nef-SPA in an E3+ backbone (vector #1) in combination with hCMV-pol-bGHpA in an E3+ backbone (vector #2), with all transgenes in the E1 parallel orientation. Fusion constructs other than the gag-pol fusion described above are also suitable for use in various divalent vaccine strategies and can be composed of any two HIV antigens fused to one another (e.g.,, nef-pol and gag-nef). These adenoviral compositions are, as above, preferably delivered along with an adenoviral composition comprising an additional HIV antigen in order to diversify the immune response generated upon administration. Therefore, a multivalent vaccine delivered in a single, or possible second, adenoviral vector is certainly contemplated as part of the present invention. Again, this mode of administration is another example of whereby an efficaceous adenovirus-based HIV-1 vaccine may be administered via a combined modality regime. It is important to note, however, that in terms of deciding on an insert for the disclosed adenoviral vectors, due consideration must be dedicated to the effective packaging limitations of the adenovirus vehicle. Adenovirus has been shown to exhibit an upper cloning capacity limit of approximately 105% of the wildtype Ad5 sequence.

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Regardless of the gene chosen for expression, it is preferred that the sequence be "optimized" for expression in a human cellular environment. A "triplet" codon of four possible nucleotide bases can exist in 64 variant forms. That these forms provide the message for only 20 different amino acids (as well as transcription initiation and termination) means that some amino acids can be coded for by more than one codon. Indeed, some amino acids have as many as six "redundant", alternative codons while some others have a single, required codon. For reasons not completely understood, alternative codons are not at all uniformly present in the endogenous DNA of differing types of cells and there appears to exist variable natural hierarchy or "preference" for certain codons in certain types of cells. As one example, the amino acid leucine is specified by any of six DNA codons including CTA, CTC, CTG, CTT, TTA, and TTG (which correspond, respectively, to the mRNA codons, CUA, CUC, CUG, CUU, UUA and UUG). Exhaustive analysis of genome codon frequencies for microorganisms has revealed endogenous DNA of E. coli most commonly contains the CTG leucine-specifying codon, while the DNA of yeasts and slime molds most commonly includes a TTA leucine-specifying codon. In view of this hierarchy, it is generally held that the likelihood of obtaining high levels of expression of a leucine-

rich polypeptide by an *E. coli* host will depend to some extent on the frequency of codon use. For example, a gene rich in TTA codons will in all probability be poorly expressed in *E. coli*, whereas a CTG rich gene will probably highly express the polypeptide. Similarly, when yeast cells are the projected transformation host cells for expression of a leucine-rich polypeptide, a preferred codon for use in an inserted DNA would be TTA.

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The implications of codon preference phenomena on recombinant DNA techniques are manifest, and the phenomenon may serve to explain many prior failures to achieve high expression levels of exogenous genes in successfully transformed host organisms—a less "preferred" codon may be repeatedly present in the inserted gene and the host cell machinery for expression may not operate as efficiently. This phenomenon suggests that synthetic genes which have been designed to include a projected host cell's preferred codons provide a preferred form of foreign genetic material for practice of recombinant DNA techniques. Thus, one aspect of this invention is an adenovirus vector or adenovirus vector in some combination with a vaccine plasmid where both specifically include a gene which is codon optimized for expression in a human cellular environment. As noted herein, a preferred gene for use in the instant invention is a codon-optimized HIV gene and, particularly, HIV gag, pol or nef.

Adenoviral vectors in accordance with the instant invention can be constructed using known techniques, such as those reviewed in Hitt et al, 1997 "Human Adenovirus Vectors for Gene Transfer into Mammalian Cells" Advances in Pharmacology 40:137-206, which is hereby incorporated by reference.

In constructing the adenoviral vectors of this invention, it is often convenient to insert them into a plasmid or shuttle vector. These techniques are known and described in Hitt et al., *supra*. This invention specifically includes both the adenovirus and the adenovirus when inserted into a shuttle plasmid.

Preferred shuttle vectors contain an adenoviral portion and a plasmid portion. The adenoviral portion is essentially the same as the adenovirus vector discussed supra, containing adenoviral sequences (with non-functional or deleted E1 and E3 regions) and the gene expression cassette, flanked by convenient restriction sites. The plasmid portion of the shuttle vector often contains an antibiotic resistance marker under transcriptional control of a prokaryotic promoter so that expression of the antibiotic does not occur in eukaryotic cells. Ampicillin resistance genes, neomycin resistance genes and other pharmaceutically acceptable antibiotic resistance markers may be used. To aid in the high level production of the polynucleotide by

fermentation in prokaryotic organisms, it is advantageous for the shuttle vector to contain a prokaryotic origin of replication and be of high copy number. A number of commercially available prokaryotic cloning vectors provide these benefits. It is desirable to remove non-essential DNA sequences. It is also desirable that the vectors not be able to replicate in eukaryotic cells. This minimizes the risk of integration of polynucleotide vaccine sequences into the recipients' genome. Tissue-specific promoters or enhancers may be used whenever it is desirable to limit expression of the polynucleotide to a particular tissue type.

In one embodiment of this invention, the pre-plasmids (e.g., pMRKAd5pol, pMRKAd5nef and pMRKAd5gag were generated by homologous recombination using the MRKHVE3 (and MRKHVO for the E3- version) backbones and the appropriate shuttle vector, as shown for pMRKAd5pol in Figure 22 and for pMRKAd5nef in Figure 23. The plasmid in linear form is capable of replication after entering the PER.C6[®] cells and virus is produced. The infected cells and media were harvested after viral replication was complete.

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Viral vectors can be propagated in various E1 complementing cell lines, including the known cell lines 293 and PER.C6[®]. Both these cell lines express the adenoviral E1 gene product. PER.C6[®] is described in WO 97/00326 (published January 3, 1997) and issued U.S. Patent No. 6,033,908, both of which are hereby incorporated by reference. It is a primary human retinoblast cell line transduced with an E1 gene segment that complements the production of replication deficient (FG) adenovirus, but is designed to prevent generation of replication competent adenovirus by homologous recombination. Cells of particular interest have been stably transformed with a transgene that encodes the AD5E1A and E1B gene, like PER.C6[®], from 459 bp to 3510 bp inclusive. 293 cells are described in Graham et al., 1977 J. Gen. Virol 36:59-72, which is hereby incorporated by reference. As stated above, consideration must be given to the adenoviral sequences present in the complementing cell line used. It is important that the sequences not overlap with that present in the vector if the possibility of recombination is to be minimized.

It has been found that vectors generated in accordance with the above description are more effective in inducing an immune response and, thus, constitute very promising vaccine candidates. More particularly, it has been found that first generation adenoviral vectors in accordance with the above description carrying a codon-optimized HIV gag gene, regulated with a strong heterologous promoter can be used as human anti-HIV vaccines, and are capable of inducing immune responses.

Standard techniques of molecular biology for preparing and purifying DNA constructs enable the preparation of the DNA immunogens of this invention.

A vaccine composition comprising an adenoviral vector in accordance with the instant invention may contain physiologically acceptable components, such as buffer, normal saline or phosphate buffered saline, sucrose, other salts and polysorbate. One preferred formulation has: 2.5-10 mM TRIS buffer, preferably about 5 mM TRIS buffer; 25-100 mM NaCl, preferably about 75 mM NaCl; 2.5-10% sucrose, preferably about 5% sucrose; 0.01 -2 mM MgCl₂; and 0.001%-0.01% polysorbate 80 (plant derived). The pH should range from about 7.0-9.0, preferably about 8.0. One skilled in the art will appreciate that other conventional vaccine excipients may also be used it make the formulation. The preferred formulation contains 5mM TRIS, 75 mM NaCl, 5% sucrose, 1mM MgCl₂, 0.005% polysorbate 80 at pH 8.0 This has a pH and divalent cation composition which is near the optimum for Ad5 stability and minimizes the potential for adsorption of virus to a glass surface. It does not cause tissue irritation upon intramuscular injection. It is preferably frozen until use.

The amount of adenoviral particles in the vaccine composition to be introduced into a vaccine recipient will depend on the strength of the transcriptional and translational promoters used and on the immunogenicity of the expressed gene product. In general, an immunologically or prophylactically effective dose of $1x10^7$ to $1x10^{12}$ particles and preferably about $1x10^{10}$ to $1x10^{11}$ particles is administered directly into muscle tissue. Subcutaneous injection, intradermal introduction, impression through the skin, and other modes of administration such as intraperitoneal, intravenous, or inhalation delivery are also contemplated. It is also contemplated that booster vaccinations are to be provided. Following vaccination with HIV adenoviral vector, boosting with a subsequent HIV adenoviral vector and/or plasmid may be desirable. Parenteral administration, such as intravenous, intramuscular, subcutaneous or other means of administration of interleukin-12 protein, concurrently with or subsequent to parenteral introduction of the vaccine compositions of this invention is also advantageous.

The adenoviral vector and/or vaccine plasmids of this invention polynucleotide may be unassociated with any proteins, adjuvants or other agents which impact on the recipients' immune system. In this case, it is desirable for the vector to be in a physiologically acceptable solution, such as, but not limited to, sterile saline or sterile buffered saline. Alternatively, the vector may be associated with an adjuvant known in the art to boost immune responses (i.e., a "biologically effective"

adjuvant), such as a protein or other carrier. Vaccine plasmids of this invention may, for instance, be delivered in saline (e.g., PBS) with or without an adjuvant. Preferred adjuvants are Alum or CRL1005 Block Copolymer. Agents which assist in the cellular uptake of DNA, such as, but not limited to, calcium ions, may also be used to advantage. These agents are generally referred to herein as transfection facilitating reagents and pharmaceutically acceptable carriers. Techniques for coating microprojectiles coated with polynucleotide are known in the art and are also useful in connection with this invention.

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This invention also includes a prime and boost regimen wherein a first adenoviral vector is administered, then a booster dose is given. The booster dose may be repeated at selected time intervals. Alternatively, a preferred inoculation scheme comprises priming with a first adenovirus serotype and then boosting with a second adenovirus serotype. More preferably, the inoculation scheme comprises priming with a first adenovirus serotype and then boosting with a second adenovirus serotype, wherein the first and second adenovirus serotypes are classified within separate subgroups of adenoviruses. The above prime/boost schemes are particularly preferred in those situations where a preexisting immunity is identified to the adenoviral vector of choice. In this type of scheme, the individual or population of individuals is primed with an adenovirus of a serotype other than that to which the preexisting immunity is identified. This enables the first adenovirus to effectuate sufficient expression of the transgene while evading existing immunity to the second adenovirus (the boosting adenovirus) and, further, allows for the subsequent delivery of the transgene via the boosting adenovirus to be more effective. Adenovirus serotype 5 is one example of a virus to which such a scheme might be desirable. In accordance with this invention, therefore, one might decide to prime with a non-group C adenovirus (e.g., Ad12, a group A adenovirus, Ad24, a group D adenovirus, or Ad35, a group B adenovirus) to evade anti-Ad5 immunity and then boost with Ad5, a group C adenovirus. Another preferred embodiment involves administration of a different adenovirus (including non-human adenovirus) vaccine followed by administration of the adenoviral vaccines disclosed. In the alternative, a viral antigen of interest can be first delivered via a viral vaccine other than an adenovirus-based vaccine, and then followed with the adenoviral vaccine disclosed. Alternative viral vaccines include but are not limited to pox virus and venezuelan equine encephilitis virus.

A large body of human and animal data supports the importance of cellular immune responses, especially CTL in controlling (or eliminating) HIV infection. In humans, very high levels of CTL develop following primary infection and correlate

with the control of viremia. Several small groups of individuals have been described who are repeatedly exposed to HIV by remain uninfected; CTL has been noted in several of these cohorts. In the SIV model of HIV infection, CTL similarly develops following primary infection, and it has been demonstrated that addition of anti-CD8 monoclonal antibody abrogated this control of infection and leads to disease progression. This invention uses adenoviral vaccines alone or in combination with plasmid vaccines to induce CTL.

The following non-limiting Examples are presented to better illustrate the invention.

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EXAMPLE 1

Removal of the Intron A Portion of the hCMV Promoter GMP grade pVIInsHIV gag was used as the starting material to amplify the hCMV promoter. PVIInsHIVgag is a plasmid comprising the CMV immediate-early (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone; see Montgomery et al., supra for a description of the plasmid backbone. The amplification was performed with primers suitably positioned to flank the hCMV promoter. A 5' primer was placed upstream of the Mscl site of the hCMV promoter and a 3' primer (designed to contain the BgIII recognition sequence) was placed 3' of the hCMV promoter. The resulting PCR product (using high fidelity Tag polymerase) which encompassed the entire hCMV promoter (minus intron A) was cloned into TOPO PCR blunt vector and then removed by double digestion with Msc1 and Bg/II. This fragment was then cloned back into the original GMP grade pV1JnsHIVgag plasmid from which the original promoter, intron A, and the gag gene were removed following Msc1 and Bg/II digestion. This ligation reaction resulted in the construction of a hCMV promoter (minus intron A) + bGHpA expression cassette within the original pV1JnsHIVgag vector backbone. This vector is designated pVIInsCMV(no intron).

The FLgag gene was excised from pV1JnsHIVgag using BgIII digestion and the 1,526 bp gene was gel purified and cloned into pV1JnsCMV(no intron) at the BgIII site. Colonies were screened using Sma1 restriction enzymes to identify clones that carried the Flgag gene in the correct orientation. This plasmid, designated pV1JnsCMV(no intron)-FLgag-bGHpA, was fully sequenced to confirm sequence integrity.

The plasmid, pV1Jns-mCMV-FLgag-bGHpA, is identical to the pV1JnsCMV(no intron)-FLgag-bGHpA except that the hCMV promoter has been removed and replaced with the murine CMV (mCMV) promoter.

Figure 3 diagrammatically shows the new transgene constructs in comparison with the original transgene.

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EXAMPLE 2

Gag Expression Assay for Modified Gag Transgenes

Gag Elisa was performed on culture supernatants obtained from transient tissue culture transfection experiments in which the two new hCMV-containing plasmid constructs, pV1JnsCMV(no intron)-FLgag-bGHpA and pV1JnsCMV(no intron)-FLgag-SPA, both devoid of intron A, were compared to pV1JnsHIVgag which, as noted above possesses the intron A as part of the hCMV promoter. Table 2 below shows the *in vitro* gag expression data of the new gag plasmids compared with the GMP grade original plasmid. The results displayed in Table 2 show that both of the new hCMV gag plasmid constructs have expression capacities comparable to the original plasmid construct which contains the intron A portion of the hCMV promoter.

Table 2: In vitro DNA transfection of original and new plasmid HIV-1 gag constructs.

Plasmid	μg gag/10e6 COS cells/5μg DNA/48 hr
HIVFL-gagPR9901a	10.8
PVIIns-hCMV-FLgag-bGHpAb	16.6
pV1Jns-hCMV-FLgag-SPA ^{bc}	12.0

^a GMP grade pV1Jns-hCMVintronA-FLgag-bGHpA.

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EXAMPLE 3

Rodent (Balb/c) Study for Modified gag Transgenes
A rodent study was performed on the two new plasmid constructs
described above – pV1JnsCMV(no intron)-FLgag-bGHpA and pV1JnsCMV(no
intron)-FLgag-SPA - in order to compare them with the construct described above
15 possessing the intron A portion of the CMV promoter, pV1JnsHIVgag. Gag antibody
and Elispot responses (described in PCT International Application No.
PCT/US00/18332 (WO 01/02607) filed July 3, 2000, claiming priority to U.S.
Provisional Application Serial No. 60/142,631, filed July 6, 1999 and U.S.
Application Serial No. 60/148,981, filed August 13, 1999, all three applications which
20 are hereby incorporated by reference) were measured. The results displayed in Table
3 below, show that the new plasmid constructs behaved equivalently to the original
construct in Balb/c mice with respect to their antibody and T-cell responses at both
dosages of plasmid DNA tested, 20 µg and 200 µg.

^b New plasmid constructions that have the intron A portion removed from the hCMV promoter.

^c In this construct the bGH terminator has been replaced with the short synthetic polyadenylation signal (SPA)

EXAMPLE 4

Table 3: HIV191: Immunogenicity of V1Jns-gag under different promoter and termination control elements.

DNA*	DNA [®] Dose,		Anti-p24 Titers (3 Wk PD1)°			SFC/10^6 Cells (4 Wk PD1) ^d		
Promoter/terminator	GMT	+SE	-SE	Media	gag197-205	p24		
HIVFL-gagPR9901	200	12800	4652	3412	2(2)	129(19)	30(11)	
(GMP grade)	20	5572	1574	1227	Ò	56(9)	25(6)	
pV1Jns-hCMV-	200	11143	2831	2257	0	98(5)	12(6)	
FL-gag-bGHpA	20	7352	2808	2032	0	73(9)	11(6)	
pV1Jns-hCMV-	200	16890	5815	4326	1(1)	94(4)	26(7)	
FL-gag-SPA	20	5971,	5361	2825	0	85(17)	38(10)	
Naïve	0	123	50	36	0	0	0	

in PBS

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Construction of the Modified Shuttle Vector - "MRKpdelE1 Shuttle"

The modifications to the original Ad5 shuttle vector (pdelE1sp1A; a vector comprising Ad5 sequences from basepairs 1-341 and 3524-5798, with a multiple cloning region between nucleotides 341 and 3524 of Ad5, included the following three manipulations carried out in sequential cloning steps as follows:

- (1) The left ITR region was extended to include the *Pac1* site at the junction between the vector backbone and the adenovirus left ITR sequences. This allow for easier manipulations using the bacterial homologous recombination system.
- 10 (2) The packaging region was extended to include sequences of the wild-type (WT) adenovirus from 342 bp to 450 bp inclusive.
 - (3) The area downstream of pIX was extended 13 nucleotides (i.e., nucleotides 3511-3523 inclusive).

These modifications (Figure 4) effectively reduced the size of the E1 deletion without overlapping with any part of the E1A/E1B gene present in the transformed PER.C6® cell line. All manipulations were performed by modifying the Ad shuttle vector pdelE1sp1A.

Once the modifications were made to the shuttle vector, the changes were incorporated into the original Ad5 adenovector backbones (pAdHVO and pAdHVE3) by bacterial homologous recombination using *E. coli* BJ5183 chemically competent cells.

bi.m. Injections into both quads, 50 μL per quad

cn=10;GMT, geometric mean titer; SE, standard. error

dn=5, pooled spleens; mean of triplicate wells and standard, deviation, in parentheses;

EXAMPLE 5

Construction of Modified Adenovector Backbones (E3+ and E3-)

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The original adenovectors pAdHVO (comprising all Ad5 sequences except those nucleotides encompassing the E1 and E3 regions) and pADHVE3 (comprising all Ad5 sequences except those nucleotides encompassing the E1 region), were each reconstructed so that they contained the modifications to the E1 region. This was accomplished by digesting the newly modified shuttle vector (MRKpdelE1 shuttle) with Pac1 and BstZ1101 and isolating the 2,734 bp fragment which corresponds to the adenovirus sequence. This fragment was co-transformed with DNA from either Cla1 linearized pAdHVO (E3- adenovector) or Cla1 linearized pAdHVE3 (E3+adenovector) into E. coli BJ5183 competent cells. At least two colonies from each transformation were selected and grown in Terrific™ broth for 6-8 hours until turbidity was reached. DNA was extracted from each cell pellet and then transformed into E. coli XL1 competent cells. One colony from each transformation was selected and grown for plasmid DNA purification. The plasmid was analyzed by restriction digestions to identify correct clones. The modified adenovectors were designated MRKpAdHVO (E3- plasmid) and MRKpAdHVE3 (E3+ plasmid). Virus from these new adenovectors (MRKHVO and MRKHVE3, respectively) as well as the old version of the adenovectors were generated in the PER.C6® cell lines to accommodate the following series of viral competition experiments. In addition, the multiple cloning site of the original shuttle vector contained ClaI, BamHI, Xho I, EcoRV, HindIII, Sal I, and Bgl II sites. This MCS was replaced with a new MCS containing Not I, Cla I, EcoRV and Asc I sites. This new MCS has been transferred to the MRKpAdHVO and MRKpAdHVE3 pre-plasmids along with the modification made to the packaging region and pIX gene.

EXAMPLE 6

Analysis of the Effect of the Packaging Signal Extension

To study the effects of the modifications made to the E1 deletion region, the viruses obtained from the original backbone (pAdHVE3) and the new backbone (MRKpAdHVE3) were mixed together in equal MOI ratios (1:1 and 5:5) and passaged through several rounds; see Figure 5, Expt.#1. Both of the viruses in the experiment contained the E3 gene intact and did not contain a transgene. The only difference between the two viruses was within the region of the E1 deletion.

Following the coinfection of the viruses at P1 (passage 1), the mixtures were propagated through an additional 4 passages at which time the cells were harvested

and the virus extracted and purified by CsCl banding. The viral DNA was extracted and digested with *Hind*III and the digestion products were then radioactively labeled. For the controls, the respective pre-plasmids (pAdHVE3 ("OLD E3+"); MRKpAdHVE3 ("NEW E3+")) were also digested with *Hind*III (and *Pac1* to remove the vector backbone) and subsequently labeled with [³³P]dATP. The radioactively labeled digestion products were subjected to gel electrophoresis and the gel was dried down onto Whatman paper before being exposed to autoradiographic film. Figure 6 clearly shows that the new adenovirus which has the addition made to the packaging signal region has a growth advantage compared with the original adenovirus. In the experiments performed (at either ratio tested), only the digestion bands pertaining to the newly modified virus were present. The diagnostic band of size 3,206 (from the new virus) was clearly present. However, there was no evidence of the diagnostic band of size 2,737 bp expected from the original virus.

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EXAMPLE 7

Analysis of the Effect of the E3 Gene

The second set of the virus competition study involved mixing equal MOI ratio (1:1) of the newly modified viruses, that obtained from MRKpAdHVO and MRKpAdHVE3 (Figure 5, Expt. #2). In this set, both viruses had the new modifications made to the E1 deletion. The first virus (that from MRKpAdHVO) does not contain an E3 gene. The second virus (that from MRKpAdHVE3) does contain the E3 gene. Neither of the viruses contain a transgene. Following coinfection of the viruses, the mixtures were propagated through an additional 4 passages at which time the cells were harvested and the total virus extracted and purified by CsCl banding. The viral DNA was extracted and digested with HindIII and the digestion products were then radioactively labeled. For the controls, the respective pre-plasmids MRKpAdHVO ("NEW E3-"); MRKpAdHVE3 ("NEW E3+") were also digested with HindIII (and Pac1 to remove the vector backbone) and then labeled with [33P]dATP. The radioactively labeled digestion products were subjected to gel electrophoresis and the gel was dried down onto Whatman paper before being exposed to autoradiographic film. Figure 6 shows the results of the viral DNA analysis of the E3+ virus and E3- virus mixing experiment. The diagnostic band corresponding to the E3+ virus (5,665 bp) was present in greater amount compared with the diagnostic band of 3,010 bp corresponding to the E3- virus. This indicates that the virus that contains the E3 gene is able to amplify more rapidly

compared with the virus that does not contain an E3 gene. This increased amplification capacity has been confirmed by growth studies; see Table 4 below.

EXAMPLE 8

Construction of the new shuttle vector containing modified gag transgene – "MRKpdelE1-CMV(no intron)-FLgag-bGHpA"

The modified plasmid pV1JnsCMV(no intron)-FLgag-bGHpA was digested with Msc1 overnight and then digested with Sfi1 for 2 hours at 50°C. The DNA was then treated with Mungbean nuclease for 30 mins at 30°C. The DNA mixture was desalted using the Qiaex II kit and then Klenow treated for 30 mins at 37°C to fully blunt the ends of the transgene fragment. The 2,559 bp transgene fragment was then gel purified. The modified shuttle vector (MRKpdelE1 shuttle) was linearized by digestion with EcoRV, treated with calf intestinal phosphatase and the resulting 6,479 bp fragment was then gel purified. The two purified fragments were then ligated together and several dozen clones were screened to check for insertion of the transgene within the shuttle vector. Diagnostic restriction digestion was performed to identify those clones carrying the transgene in the E1 parallel and E1 anti-parallel orientation. This strategy was followed to clone in the other gag transgenes in the MRKpdelE1 shuttle vector.

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EXAMPLE 9

Construction of the MRK FG Adenovectors

The shuttle vector containing the HIV-1 gag transgene in the E1 parallel orientation, MRKpdelE1-CMV(no intron)-FLgag-bGHpA, was digested with Pac1. 25 The reaction mixture was digested with BsfZ171. The 5,291 bp fragment was purified by gel extraction. The MRKpAdHVE3 plasmid was digested with Cla1 overnight at 37°C and gel purified. About 100 ng of the 5,290 bp shuttle +transgene fragment and ~100 ng of linearized MRKpAdHVE3 DNA were co-transformed into E. coli BJ5183 chemically competent cells. Several clones were selected and grown in 2 ml 30 Terrific™ broth for 6-8 hours, until turbidity was reached. The total DNA from the cell pellet was purified using Qiagen alkaline lysis and phenol chloroform method. The DNA was precipitated with isopropanol and resuspended in 20 µl dH₂0. A 2 µl aliquot of this DNA was transformed into E. coli XL-1 competent cells. A single colony from each separate transformation was selected and grown overnight in 3 ml LB +100 μg/ml ampicillin. The DNA was isolated using Qiagen columns. A positive 35 clone was identified by digestion with the restriction enzyme BstEII which cleaves

within the gag gene as well as the plasmid backbone. The pre-plasmid clone is designated MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA and is 37,498 bp in size. This strategy was followed to generate E3- and E3+ versions of each of the other gag transgene constructions in both E1 parallel and E1 anti-parallel versions. Figures 7A, 7B and 7C show the various combinations of adenovectors constructed.

EXAMPLE 10

Plasmid Competition Studies

A series of plasmid competition studies was carried out. Briefly, the screening of the various combinations of new constructs was performed by mixing equal amounts of each of two competing plasmids. In the experiment shown in Figure 8A, plasmids containing the same transgene but in different orientations were mixed together to create a "competition" between the two plasmids. The aim was to look at the effects of transgene orientation. In the experiment shown in Figure 8B, plasmids containing different polyadenylation signals (but in the same orientation) were mixed together in equal amounts. The aim was to assess effects of polyA signals. Following the initial transfection, the virus was passaged through ten rounds and the viral DNA analyzed by radioactive restriction analysis.

Analysis of the viral species from the plasmid mixing experiment (Figure 8A) showed that adenovectors which had the transgene inserted in the E1 parallel orientation amplified better and were able to out-compete the adenovirus which had the transgene inserted in the E1 anti-parallel orientation. Viral DNA analysis of the mixtures at passage 3 and certainly at passage 6, showed a greater ratio of the virus carrying the transgene in the E1 parallel orientation compared with the E1 antiparallel version. By passage 10, the only viral species observed was the adenovector with the transgene in the E1 parallel orientation for both transgenes tested (hCMV(no intron)-FLgag-bGHpA and hCMV(no intron)-FLgag-SPA).

Analysis of the viral species from the plasmid mixing experiment #2 (Figure 8B) at passages 3 and 6 showed that the polyadenylation signals tested (bGHpA and SPA) did not have an effect on the growth of the virus. Even at passage 10 the two viral species in the mixture were still present in equal amounts.

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EXAMPLE 11

Virus generation of an enhanced adenoviral construct - "MRK Ad5 HIV-1gag"

The results obtained from the competition study allowed us to make the following conclusions: (1) The packaging signal extension is beneficial; (2) Presence of E3 does enhance viral growth; (3) E1 parallel orientation is recommended; and (4) PolyA signals have no effect on the growth of the adenovirus.

MRK Ad5 HIV-1 gag exhibited the most desirable results. This construct contains the hCMV(no intron)-FLgag-bGHpA transgene inserted into the new E3+adenovector backbone, MRKpAdHVE3, in the E1 parallel orientation. We have designated this adenovector MRK Ad5 HIV-1 gag. This construct was prepared as outlined below:

The pre-plasmid MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA was digested was Pac1 to release the vector backbone and 3.3 µg was transfected by calcium phosphate method (Amersham Pharmacia Biotech.) in a 6 cm dish containing PER.C6® cells at ~60% confluence. Once CPE was reached (7-10 days), the culture was freeze/thawed three times and the cell debris pelleted. 1 ml of this cell lysate was used to infect into a 6 cm dish containing PER.C6[®] cells at 80-90% confluence. Once CPE was reached, the culture was freeze/thawed three times and the cell debris pelleted. The cell lysate was then used to infect a 15 cm dish containing PER.C6[®] cells at 80-90% confluence. This infection procedure was continued and expanded at passage 6. The virus was then extracted from the cell pellet by CsCl method. Two bandings were performed (3-gradient CsCl followed by a continuous CsCl gradient). Following the second banding, the virus was dialyzed in A105 buffer. Viral DNA was extracted using pronase treatment followed by phenol chloroform. The viral DNA was then digested with *Hind*III and radioactively labeled with [33P]dATP. Following gel electrophoresis to separate the digestion products the gel was dried down on Whatman paper and then subjected to autoradiography. The digestion products were compared with the digestion products from the pre-plasmid (that had been digested with Pac1/HindIII prior to labeling). The expected sizes were observed, indicating that the virus had been successfully rescued. This strategy was used to rescue virus from each of the various adenovector plasmid constructs prepared.

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EXAMPLE 12

Stability Analyses

To determine whether the various adenovector constructs (e.g., MRK Ad5 HIV-1 gag) show genetic stability, the viruses were each passaged continually. The viral DNA was analyzed at passages 3, 6 and 10. Each virus maintained its correct genetic structure. In addition, the stability of the MRK Ad5 HIV-1 gag was analyzed under propagation conditions similar to that performed in large scale production. For this analysis, the transfections of MRK Ad5 HIV-1 gag as well as three other adenoviral vectors were repeated and the virus was purified at P3. The three other adenovectors were as follows: (1) that comprising hCMV(no intron)-Flgag with a bGHpA terminator in an E3- adenovector backbone; (2) that comprising hCMV(no intron)-Flgag with a SPA termination signal in an E3+ adenovector backbone, and that comprising a mCMV-Flgag with a bGHpA terminator in an E3+ adenovector backbone. All of the vectors have the transgene inserted in the E1 parallel orientation. Viral DNA was analyzed by radioactive restriction analysis to confirm that it was correct before being delivered to fermentation cell culture for continued passaging in serum-free media. At P5 each of the four viruses were purified and the viral DNA extracted for analysis by the restriction digestion and radiolabeling procedure. This virus has subsequently been used in a series of studies (in vitro gag expression in COS cells, rodent study and rhesus monkey study) as will be described below. The viruses from P5 are shown in Figure 9.

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The passaging under serum-free conditions was continued for the MRKHVE3 (transgene-less, obtained from MRKpAdHVE3 pre-plasmid) and the MRKAd5HIV-1gag (obtained from MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA pre-plasmid) viruses. Figure 10 shows viral DNA analysis by radioactive restriction digestion at passage 11 for MRKHVE3, MRKAd5HIV-1gagE3-, and passage 11 and 12 for MRKAd5HIV-1gag. Aside from the first lane which is the DNA marker lane, the next three lanes are virus from the pre-plasmid controls (controls based on the original virus) - MRKpAdHVE3 (also referred to as "pMRKHVE3"), MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA, and pMRKAd5gag(E3-), respectively. As seen in Figure 10, each of the viral DNA samples show the expected bands with no extraneous bands showing. This signifies that there are no major variant adenovirus species present that can be detected by autoradiography.

Figure 11 shows the results of viral competition study between MRKHVE3 and MRKAd5HIV-1gag. These viruses were mixed together at equal MOI (140 viral

particles each; 280 vp total) at passage 6 and continued to be passaged until P11. Aside from the first lane which is the DNA marker lane, the next two lanes are the pre-plasmid controls obtained from MRKpAdHVE3 and MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA. The next two lanes are the viral DNA from the starting viral material at passage six. The last two lanes are the competition studies performed in duplicate. The data in Figure 11 shows the effect the gag transgene in culture. Growth of a MRKAd5gag virus was compared with growth of a "transgene-less" MRKHVE3. These two viruses were infected at the same MOI (i.e. 140 vp each) at passage 6 and then passaged through to passage 11 and the viral pool was analyzed by radioactive restriction analysis. The data shows that one virus did not out compete the other. Therefore, the gag transgene did not show obvious signs of toxicity to the adenovirus.

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Analysis by *Hind*III digestion shows that each virus specie is present in approximately equal amounts. As above, there does not appear to be signs of any extraneous bands. Figure 12 shows higher passage numbers for MRKAd5HIV-1gag grown under serum-containing conditions. The genome integrity again has been maintained and there is no evidence of rearrangements, even at the highest passage level (P21).

Each of the four vectors shown in Figure 9 were analyzed for amplification capacity. Table 4 below shows the QPA analysis used in the estimation of viral amplification ratios at P4. The determination of the amplification ratio for the original HIV-1 gag construct is based on the clinical lot at P12. It has been shown that amplification rates increases with higher passage number for the original virus. The reason for this observation is due to the emergence of variants which exhibit increased growth rates compared to the intact adenovector. With continued passaging of the original Ad gag vector, the level of variants increases and hence amplification rates increase also.

The MRK Ad5 HIV-1 gag virus has also been continually passaged under process conditions (i.e., serum-free media). Viral DNA extracted from passages 11 and 12 show no evidence of rearrangement.

Table 4:
Amplification Ratios Based on AEX and QPA Analysis of Virus Amplification from Passage 3 to Passage 4.

Ad gag construct	Amplification Ratio			
MRKAd5gag	470			
HCMV-Flgag-bGHpA [E3-]	115			
HCMV-Flgag-SPA [E3+]	320			
mCMV-FLgag-bGHpA [E3+]	420			
Original construct *	40 - 50			

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EXAMPLE 13

Analytical Evaluation of the enhanced Ad5 Constructs

To study the effects of the transgene and the E3 gene on virus amplification, the enhanced adenoviral vector, MRK Ad5 HIV-1 gag, along with its transgene-less version (MRKpAdHVE3) and its E3- version (MRK Ad5 HIV-1 gag E3-), was studied for several passages under serum-free conditions. Table 5A shows the amplification ratios determined for passages P3 to P8 for MRK Ad5 HIV-1 gag. Within a certain MOI range, it has been determined that the virus output is directly proportional to the virus input. Therefore, the greater the number of virus particles per cell at infection, the greater the virus amount produced. Viral amplification ratios, on the other hand, are inversely proportional to the virus input. The lower the virus input, the greater the amplification ratio.

Table 5B shows the amplification rates of the new E3+ vector backbone MRKpAdHVE3. It has a significantly lower rate of amplification compared with the gag transgene containing version. This may be contributed to the larger size MRK Ad5 HIV-1 gag since it contains the transgene. This inclusion of the transgene brings the size of the adenovirus closer to the size of a wild type Ad5 virus. It is well known that adenoviruses amplify best when they are at close to their wild type genomic size.

^{*} This estimation is based on the clinical lot growth characteristics at Passage 12.

Wild type Ad5 is 35,935 bp. The MRKpAdHVE3 is 32, 905 bp in length. The enhanced adenovector MRK Ad5 HIV-1 gag is 35,453bp (See Figure 14 for vector map; see also Figure 15A-X show the complete pre-adenoviral vector sequence, which includes an additional 2,021 bp of the vector backbone).

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Table 5C shows the amplification rates of the new E3- gag containing virus MRK Ad5 HIV-1 gag E3-. Once again, this virus shows lower growth rate than the enhanced adenoviral vector. This may be attributed to the decreased sized of this virus (due to the E3 gene deletion) compared with wild type Ad5. The MRK Ad5 HIV-1 gag E3- virus is 32,810 bp in length. This can be compared with the wild type Ad5 which is 35,935 bp and MRK Ad5 HIV-1 gag which is 35,453 bp in length.

Table 5A: Amplification ratios determined by AEX and QPA for MRKAd5gag over several continuous passaging in serum free media. Following P5, two replicate samples were taken (rep-1 and rep-2) and analyzed.

MRKAd5gag rep1

	XV (10 ⁵ calls/n Intection	I), Visibility (%) Harvest	Harvest Time	Cell Passage Number	Titler 10 ^{co} vp/mi cultura	Tear 10" vp/cet	OPA 10° TCID _{so} /mi	Ratio AEXXIPA	Amplification Ratio	AEX Imamal Control
P4	1,49, 81%	0.58, 50%	44	46	8.7	5.9	1.72	50	470 (MOI = 125)	
P5	1,38, 93%	0.66, 47%	48	49	6,7	4.9	1.38	49	170	
PB	1.04, 94%	0.68, 77%	47	48	5.8	5.6	1.42	41	200	
P7	1.50, 84%	0.95, 61%	49.5	50	3.9	1.4	0.97	40	50	1
P7	1.09, 97%	0.76, 59%	50	52	5.2	4.7	1.70	81	170	1
PB	1.03, 94%	0.86, 64%	47.5	64	9.0	8.7	1.10	82	310	
P9	0,89,95%	0.99, 73%	47.5	58	4,4	4.9	1.03	43	175	3.12 2.84
P10	1,09, 91%	1.06, 65%	47.5	58	8.0	2.8	1.16	26	100	2.70 2.60
PII	1.19, 88%	0.98, 65%	47	60	3.6	3.0	1.15	31	110	2.70 2.70
P12	0.98, 91%	0.85, 63%	47.5	47	5.4	6.5	1.20	45	200	2.88 2.60
P13	1,00, 88%	0,70, 57%	49	49	5.8	5.8	1.11	52	210	3.18 3.18
P14	1.94, 82%	0.88, 67%	46 .	53	8.6	4.4			160	3.2B 3.27
P15	0.57, 96%	0.64, 66%	47	47	6.9	7.1			250	3.12 2.91

Table 5B: Amplification ratios determined by AEX and QPA for MRKHVE3 over several continuous passaging in serum free media. MRKHVE3 is the new vector backbone which does NOT carry a transgene.

MRKHVE3

	Xv (10° celts/n Infection	ni), Viability (%) Harvest	Harvest Time	Cell Passage Number	Ther 10 ¹⁰ vp/ml culture	Titler 10° vp/cell	QPA 10" TCID ₈₀ /ml	Ratio AEX:QPA	Amplification Ratio	AEX Internal Control
P4	1.10, 97%	1.28, 79%	49	54	4.1	3.8	1.70	25	300 (MOI = 125)	
P5	0.92, 89%	1.18, 77%	47	. 48	4.3	4.7	1.24	35	170	
P6	1,55, 86%	1,26, 76%	49.5	50	12	0.8	0.58	21	30	
P6	1.09, 97%	1.11,81%	49	52	4.0	3.6	1.16	34	130	
P7	1.17, 91%	1,22, 91%	47.5	54	3.7	3.2	0.50	74	110	
P8	0.98, 88%	1,41, 83%	48	58	21	21	0.47	45	75	3.12 2.64
PB	1,20, 89%	1.26, 81%	47.5	58	0.8	0.7	0.29	28	25	2.70 2.60
P10	0.99, 82%	1.55, 85%	47	60	23	23	0.43	. 53	80	2.70 2.70
PII	1,07,96%	1,25, 83%	48	47	2.7	2.5	0.41	66	90	2.86 2.60
P12	0.80, 91%	1.14, 80%	49.5	49	5.9	7.4	0.48	123	250	3.18
P13	1,98,95%	1.14, 85%	45.5	53	5.8	3.0			110	3.28 3.27
P14	0.97, 95%	1.03, 98%	48.5	47	9.4	8.7			350	3.12 2.91
P15	0.87, 99%	0.97, 89%	49.5	49	5.3	6.1			218	2.78 2.52

Table 5C. Amplification ratios determined by AEX and QPA for MRKAd5gag(E3-) over several continuous passaging in serum free media. This construct is identical to the MRKAd5gag construct except that this version is DELETED of the E3 gene.

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MRKAd5gag(E3-)

	Xv (10° cells/r Infection	ni), Visbility (%) Harvest	Harvest Time h.p.i.	Cell Passage Number	Titler 10 ¹⁰ vp/ml culture	Titer 10° vp/ceti	QPA 10° TCID _{ES} And	Ratio AEX:QPA	Amplification Ratio	AEX Internal Contro
P4	1.62, 77%	1.12, 62%	47.5	48	2.0	1.2	0.52	20	100 (MOI=125)	
P5	1.16, 92%	0.62, 43%	49	49	3.3	2.9	0.99	34	100	
P6	1.71, 85%	0.20, 10%	49	50	4.7	2.7	1.70	28	100	
P6	1.09, 97%	0.63, 54%	49.5	52	5.4	5.0	1.76	31	180	
P7	1.17, 91%	0.98, 72%	47.50	54	7.1	6.1	0.67	106	220	-
P8	0.98, 88%	0.77, 48%	48	56	8.1	3.2	0.66	47	115	3.12
P9	1.20, 89%	1.03, 72%	48	58	1.8	1.5	0.57	32	55	2.84 2.70
P10	0.99, 82%	0.80, 62%	46.5	60	3.2	3.2	69.0	47	115	2.60 2.70
P11	1.07, 96%	0.88, 70%	48.5	47	5.9	5.5	0.68	87	200	2.70 2.88
P12	0.80, 91%	0.57, 59%	50	49	£.1	6.4	0.72	71	230	2.60 3.18
P13	1.96, 95%	0.91, 59%	45.5	53	7.4	3.8			135	3.18 · 3.28
P14	0.97, 96%	0.81, 74%	48	47	6.8	7.0			250	3.27 3.12
P15	0.87, 99%	0.84, 56%	49	49	4.8	5.5			196	2.91 2.78 2.52

EXAMPLE 14

Gag Expression Analysis of the Novel Constructs

In vitro gag analysis of the MRK Ad5 HIV-1 gag and the original HIV-gag vectors (research and clinical lot) show comparable gag expression. The clinical lot shows only a slightly reduced gag expression level. The most noticeable difference is with the mCMV vector. This vector shows roughly 3 fold lower expression levels compared with the other vectors tested (which all contain hCMV promoters). The mCMV-FLgag with bGHpA assay was performed three times using different propagation and purification lots and it consistently exhibited weaker gag expression.

EXAMPLE 15

Evaluation of MRK Ad5 HIV-1 gag and Other gag-Containing Adenovectors in Balb/c Mice

Cohorts of 10 balb/c mice were vaccinated intramuscularly with escalating doses of MRK Ad5 HIV-1 gag, and the research and clinical lots of original Ad5HIV-1gag. Serum samples were collected 3 weeks post dose 1 and analyzed by anti-p24 sandwich ELISA.

Anti-p24 titers in mice that received MRK Ad5 HIV-1 gag (107 and 109 vp(viral particle) doses) were comparable (Figure 13) to those of the research lot of Ad5HIV-1 gag, for which much of the early rhesus data were generated on. These titers were also comparable when E3 is deleted (MRKAd5hCMVgagbGHpA(E3-)) or SPA is substituted for bGHpA terminator (MRKAd5 hCMV-gag-SPA (E3+)) or murine CMV promoter is used in place of hCMV (MRKAd5 mCMV-gag-bGHpA (E3+)) in the MRKAd5 backbone.

The results shown in Table 7 indicate that the three other vectors (in addition to the preferred vector, MRK Ad5 HIV-1 gag, are also capable of inducing strong anti-gag antibody responses in mice. Interestingly enough, while the mCMV-FLgag construct containing bGHpA and E3+ in an E1 parallel orientation showed lowest gag expression in the COS cell *in vitro* infection (Table 6) in comparison with the other vectors tested, it generated the greatest anti-gag antibody response this *in vivo* Balb/c study. Table 7 also shows a dose response in anti-gag antibody production in both the research and the clinical lot. As expected, the clinical lot shows reduced anti-gag antibody induction at each dosage level compared to the same dosage used for the research lot.

Table 6: In vitro analysis for gag expression in COS cells by Elisa assay.

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Viral Vectors ^a	µg gag/4.8x10e5 COS/10e8 parts/48hr
MRKAd5gag ^b	1.40
Clinical lot Ad5gag ^c	1.28
Research lot Ad5gag ^d	1.32
MCMVFL-gagbGHpA ^c	0.42

^a A_{260mm} absorbance readings taken for viral particle determinations.

^b MRKAd5gag was produced in serum free conditions and purified at P5.

^cClinical lot# Ad5gagFN0001

⁵ d Research Ad5FLgag lot# 6399

[°] mCMVFL-gagbGHpA was produced in serum free conditions and purified at P5.

Table 7: mHIV020 Anti-p24 Ab Titers in Balb/c mice (n=10) vaccinated with various Adgag constructs and lots (3 week post dose1).

Group ID	Vaccine	Dose (vp)	GMT	SE upper	SE lower
1	^a MRKAd5gag	10^7	25600	5877	4780
2	B	10^9	409600	94028	76473
3	hCMV FL-gag bGHpA [E3-] →	10^7	7352	2077	1620
4		10^9	235253	59767	47659
5	hCMV FL-gag SPA [E3+] →	10^7	12800	9905	236
6		10^9	310419	99181	75165
7	bmCMV FL-gag bGHpA [E3+] →	10^7	44572	23504	15389
8	•	10^9	941014	239068	190836
9	^c hCMV FL-gag bGHpA [E3-] ←	10^7	3676	934	745
10	•	10^9	117627	17491	15227
11	research lot hCMV intronA FL-gag bGHpA [E3-] <-	10^6	528	262	175
12		10^7	14703	5274	3882
13	" "	10^8	58813	14942	11915
14		10^9	204800	53232	42250
15	clinical lot hCMVintronA FL-gag bGHpA [E3-] <-	10^6	230	82	61
16		10^7	4222	3405	1138
17		10^8	19401	3939	3274
18	3	10^9	89144	25187	19639
19	Naïve	none	93	7	. 6

*2x50 µL i.m. (quad) injections/animal .

P.I.s: Youil, Chen, Casimiro

Vaccination: T. Toner, Q. Su

Assay: M. Chen

EXAMPLE 16

Comparison of Humoral and Cellular Responses Towards the Original Ad-gag Construct with the New MRK Ad5 HIV-1 gag in Rhesus Monkeys

- Cohorts of 3 rhesus monkeys were vaccinated intramuscularly with MRK Ad5 5 HIV-1 gag or the clinical Ad5gag bulk at two doses, 10^{11} vp and 10^9 vp. Immunizations were conducted at week 0, 4, and 25. Serum and PBMC samples were collected at selected time points. The serum sample were assayed for anti-p24 Ab titers (using competitive based assay) and the PBMCs for antigen-specific IFNgamma secretion following overnight stimulation with gag 20-mer peptide pool (via
- 10 ELISpot assay).

The results shown in Table 8 indicate comparable responses with respect to the generation of anti-gag antibodies. The frequencies of gag-specific T cells in

^aThe structure of MRKAd5gag is: hCMVFL-gagbGHpA [E3+] → The same lot of MRKAd5gag used in this rodent study was used in the Rhesus monkey study (Tables 7 and 8).

^bThe same lot of mCMVFL-gagbGHpA[E3+] used in the in vitro study (Table 6) ws used here.

^cThis construct was designed by Volker Sandig. It contains a shorter version of the hCMV promoter than that used in the MRK constructs. The adenovector backbone is identical to the original backbone used in the original Adgag vector. Expression at 10e7 dose from this vector is 7 fold lower then the same dose of the MRKAd5gag and 4 fold lower than the research lot.

peripheral blood assummarized in Table 9 demonstrate a strong cellular immune response generated after a single dose with the new construct MRK Ad5 HIV-1 gag. The responses are also boostable with second dose of the same vector. The vector is also able to induce CD8+ T cell responses (as evident by remaining spot counts after CD4+ depletion of PBMCs) which are responsible for cytotoxic activity.

Table 8 Anti-p24 antibody titers (in mMU/mL) in rhesus macaques immunized with

gag-expressing adenovectors (Protocol HIV203).

Vaccine	Pre	Wk4	Wk8	Wk 12	Wk 16	Wk 20	Wk 25	Wk 28
MRKAd5gag°, 10^11 vp								
97N010	<10	118	5528	11523	7062	21997	ND	51593
97N116	<10	62	772	1447	1562	2174	ND	20029
98X007	<10	66.	3353	6156	6845	3719	ND_	24031
MR KAd5gog, 10^9 vp								
97N120	<10	51	204	318	366	482	ND	6550
97N144	<10	18	118_	274	706	888	ND	7136
98X008	<10	15	444	386	996	1072	ND	12851
Ad5gag ^b , Clinical Lat, 10^11 vp								
97X001	<10	87	2579	4718	7174	7250	ND	69226
97N146	<10	72	3604_	7380	7526	18906	ND	60283
98X009	<10	78	4183	3946	3124	6956	ND	26226
Ad5gag, Clinical Lot, 10^9 vp								
97N020	<10	<10	143	371	390	1821	ND	17177
97X003	<10	<10	39	93	156	596	ND	2053
98X012	<10	81	342	717	956	1558	ND	11861
MRKActigat (hCMV, bGHpA, E3+)					<u> </u>		.	
Pariginal Actigag vector (hCMV/Intro	n A bGHp	A. E3-), lot	#FN0001_					
ND, not determined		1		1	<u> </u>	<u> </u>	<u></u>	

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Table 9. Number of gag-specific T cells per million peripheral blood mononuclear cells (PBMCs) in rhesus monkeys immunized with gag-expressing adenovectors. Also included are those frequencies in PBMCs depleted of CD4⁺ T cells.

Grp 0	Vaccination	Monkey ID	1=4		1=6	Wk		Wk	I=1	Wk	T ₽2	Wk	T=2	Wk
	T=0,4,25 wks		Media	Gog H ^b	Media	Gog H	Medio	Gog H	Media	Goog H	Media	Gog H	Media	Gog H
									_					
1	MRKAC5000	97ND1D	В	89	0	395	0	1058	0	1174	3	775	4	1074
	1041 VP	97N010(CD4-)	4	38	. '		3	993	. !		0	76	0	594
		97N116	1 1	398	1	609	0	534	4	395	1	261	0	408
		97N116(CD4-)	11	676			0	593	_ 1		0	184	0	666
		98X007	10	579	0	1304	3	2193	וו	2118	3	1588	0	2113
		98X007(CD4-)	20	965		i	0	2675	l		0	1656	0	1278
2	MRKAdicco	97N120	5	275	1	249	4	141	4	119	9	206	4	219
_	10/9 VD	97N120(CD4-)	11	170			0	85	1		0	75	1	219
	•	97N144	3	235	6	438	1	318	3	256	1	98	5	373
		97N144(CD4-)	6	148	1		0	285			ND	NO	0	625
		98X008	4	368	1	1090	3	891	4	673	3	473	5	735
		98X008(CD4-)	14	696	1		0	1175	1	1	٥	391	4	848
3	AdSgoog clinical lat	97X001	<u> </u>	261	1	485	0	817	0	12200	1	894	0	1858
•	10/11 VD	97X001(CD4-)	10	283	1 •		3	996	l	1	0	1010	0	112
		97N146	3	150	1 1	465	0	339	1	1272	3	1238	3	178
		97N146(CD4-)	ها	133	(l	lo	370	l	l	0	654	0	971
		980009	0	93	1 3	339	3	559	0	896	1	384	0	174
		98X009(CD4-)	0	73			0	333			0	225	0	600
4	Actions dinical lat	97N020	3	30	1	101	0	8	0	36	0	26	0	41
	10'9 vp	97N020(CD4-)	10	29	1	I	0	15	ĺ	1	0	1	0	16
		970003	4	68	5	134	0	18	1	38	4	38	6	81
		97X003(CD4-)	9	40	1		0	٥	1		٥	4	0	19
		98X012	5	95	3	54	1	34	0	18	0	20	1 1	121
		98X012(CD4-)	11	70	l		0	11		1	٥	В	0	41
5	Nave	96RD41	6	8	1	1	0	0	0	0	0	0	1	0
		053F	14	18	5	16.	20	14	19	15	10	15	24	°

Based on either 4x10/5 or 2x10/5 celts per well (depending on spot density)

ND, not determined

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"mock or no pectide control

Pool of 20-corpections overlapping by 10 corond encompositing the good sequence

The adenovectors described herein and, particularly, MRK Ad5 HIV-1 gag, represent very promising HIV-gag adenovectors with respect to their enhanced growth characteristics in both serum and, more importantly, in serum-free media conditions. In comparison with the current HIV-1 gag adenovector construct, MRK Ad5 HIV-1 gag shows a 5-10 fold increased amplification rate. We have shown that it is genetically stable at passage 21. This construct is able to generate significant cellular immune responses in vivo even at a relatively low dose of 10^9 vp. The potency of the MRKAd5gag construct is comparable to, if not better than the original HIV-1gag vector as shown in this rhesus monkey study.

EXAMPLE 17 CODON OPTIMIZED HIV-1 POL AND CODON OPTIMIZED HIV-1 POL MODIFICATIONS

The open reading frames for the various synthetic *pol* genes disclosed herein comprise coding sequences for the reverse transcriptase (or RT which consists of a polymerase and RNase H activity) and integrase (IN). The protein sequence is based

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on that of Hxb2r, a clonal isolate of IIIB; this sequence has been shown to be closest to the consensus clade B sequence with only 16 nonidentical residues out of 848 (Korber, et al., 1998, Human retroviruses and AIDS, Los Alamos National Laboratory, Los Alamos, New Mexico). The skilled artisan will understand after review of this specification that any available HIV-1 or HIV-2 strain provides a potential template for the generation of HIV pol DNA vaccine constructs disclosed herein. It is further noted that the protease gene is excluded from the DNA vaccine constructs of the present invention to insure safety from any residual protease activity in spite of mutational inactivation. The design of the gene sequences for both wildtype (wt-pol) and inactivated pol (IA-pol) incorporates the use of human preferred ("humanized") codons for each amino acid residue in the sequence in order to maximize in vivo mammalian expression (Lathe, 1985, J. Mol. Biol. 183:1-12). As can be discerned by inspecting the codon usage in SEQ ID NOs: 1, 3, 5 and 7, the following codon usage for mammalian optimization is preferred: Met (ATG), Gly (GGC), Lys (AAG), Trp (TGG), Ser (TCC), Arg (AGG), Val (GTG), Pro (CCC), Thr (ACC), Glu (GAG); Leu (CTG), His (CAC), Ile (ATC), Asn (AAC), Cys (TGC), Ala (GCC), Gln (CAG), Phe (TTC) and Tyr (TAC). For an additional discussion relating to mammalian (human) codon optimization, see WO 97/31115 (PCT/US97/02294), which, as noted elsewhere in this specification, is hereby incorporated by reference. It is intended that the skilled artisan may use alternative versions of codon optimization or may omit this step when generating HIV pol vaccine constructs within the scope of the present invention. Therefore, the present invention also relates to non-codon optimized versions of DNA molecules and associated recombinant adenoviral HIV vaccines which encode the various wild type and modified forms of the HIV Pol protein disclosed herein. However, codon optimization of these constructs is a preferred embodiment of this invention.

A particular embodiment of this portion of the invention comprisies codon optimized nucleotide sequences which encode wt-pol DNA constructs (herein, "wt-pol" or "wt-pol (codon optimized))" wherein DNA sequences encoding the protease (PR) activity are deleted, leaving codon optimized "wild type" sequences which encode RT (reverse transcriptase and RNase H activity) and IN integrase activity. A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:1, the open reading frame being contained from an initiating Met residue at nucleotides 10-12 to a termination codon from nucleotides 2560-2562. SEQ ID NO:1 is as follows:

AGATCTACCA TGGCCCCCAT CTCCCCCATT GAGACTGTGC CTGTGAAGCT GAAGCCTGGC

ATGGATGGCC CCAAGGTGAA GCAGTGGCCC CTGACTGAGG AGAAGATCAA GGCCCTGGTG

	GAAATCTGCA	CTGAGATGGA	GAAGGAGGC	AAAATCTCCA	AGATTGGCCC	CGAGAACCCC
	TACAACACCC	CTGTGTTTGC	CATCAAGAAG	AAGGACTCCA	CCAAGTGGAG	GAAGCTGGTG
	GACTTCAGGG	AGCTGAACAA	GAGGACCCAG	GACTTCTGGG	AGGTGCAGCT	GGGCATCCCC
	CACCCCGCTG	GCCTGAAGAA	GAAGAAGTCT	GTGACTGTGC	TGGATGTGGG	GGATGCCTAC
5	TTCTCTGTGC	CCCTGGATGA	GGACTTCAGG	AAGTACACTG	CCTTCACCAT	CCCCTCCATC
	AACAATGAGA	CCCCTGGCAT	CAGGTACCAG	TACAATGTGC	TGCCCCAGGG	CTGGAAGGGC
	TCCCCTGCCA	TCTTCCAGTC	CTCCATGACC	AAGATCCTGG	AGCCCTTCAG	GAAGCAGAAC
	CCTGACATTG	TGATCTACCA	GTACATGGAT	GACCTGTATG	TGGGCTCTGA	CCTGGAGATT
	GGGCAGCACA	GGACCAAGAT	TGAGGAGCTG	AGGCAGCACC	TGCTGAGGTG	GGGCCTGACC
10	ACCCCTGACA	AGAAGCACCA	GAAGGAGCCC	CCCTTCCTGT	GGATGGGCTA	TGAGCTGCAC
	CCCGACAAGT	GGACTGTGCA	GCCCATTGTG	CTGCCTGAGA	AGGACTCCTG	GACTGTGAAT
	GACATCCAGA	AGCTGGTGGG	CAAGCTGAAC	TGGGCCTCCC	AAATCTACCC	TGGCATCAAG
	GTGAGGCAGC	TGTGCAAGCT	GCTGAGGGGC	ACCAAGGCCC	TGACTGAGGT	GATCCCCCTG
	ACTGAGGAGG	CTGAGCTGGA	GCTGGCTGAG	AACAGGGAGA	TCCTGAAGGA	GCCTGTGCAT
15	GGGGTGTACT	ATGACCCCTC	CAAGGACCTG	ATTGCTGAGA	TCCAGAAGCA	GGGCCAGGGC
	CAGTGGACCT	ACCAAATCTA	CCAGGAGCCC	TTCAAGAACC	TGAAGACTGG	CAAGTATGCC
	AGGATGAGGG	GGGCCCACAC	CAATGATGTG	AAGCAGCTGA	CTGAGGCTGT	GCAGAAGATC
	ACCACTGAGT	CCATTGTGAT	CTGGGGCAAG	ACCCCCAAGT	TCAAGCTGCC	CATCCAGAAG
	GAGACCTGGG	AGACCTGGTG	GACTGAGTAC	TGGCAGGCCA	CCTGGATCCC	TGAGTGGGAG
20	TTTGTGAACA	CCCCCCCT	GGTGAAGCTG	TGGTACCAGC	TGGAGAAGGA	GCCCATTGTG
	GGGGCTGAGA	CCTTCTATGT	GGATGGGGCT	GCCAACAGGG	AGACCAAGCT	GGGCAAGGCT
	GGCTATGTGA	CCAACAGGGG	CAGGCAGAAG	GTGGTGACCC	TGACTGACAC	CACCAACCAG
	AAGACTGAGC	TCCAGGCCAT	CTACCTGGCC	CTCCAGGACT	CTGGCCTGGA	GGTGAACATT
	GTGACTGACT	CCCAGTATGC	CCTGGGCATC	ATCCAGGCCC	AGCCTGATCA	GTCTGAGTCT
25	GAGCTGGTGA	ACCAGATCAT	TGAGCAGCTG	ATCAAGAAGG	AGAAGGTGTA	CCTGGCCTGG
	GTGCCTGCCC	ACAAGGGCAT	TGGGGGCAAT	GAGCAGGTGG	ACAAGCTGGT	GTCTGCTGGC
	ATCAGGAAGG	TGCTGTTCCT	GGATGGCATT	GACAAGGCCC	AGGATGAGCA	TGAGAAGTAC
	CACTCCAACT	GGAGGGCTAT	GGCCTCTGAC	TTCAACCTGC	CCCCTGTGGT	GGCTAAGGAG
	ATTGTGGCCT	CCTGTGACAA	GTGCCAGCTG	AAGGGGGAGG	CCATGCATGG	GCAGGTGGAC
30	TGCTCCCCTG	GCATCTGGCA	GCTGGACTGC	ACCCACCTGG	AGGGCAAGGT	GATCCTGGTG
	· GCTGTGCATG	TGGCCTCCGG	CTACATTGAG	GCTGAGGTGA	TCCCTGCTGA	GACAGGCCAG
	GAGACTGCCT	ACTTCCTGCT	GAAGCTGGCT	GGCAGGTGGC	CTGTGAAGAC	CATCCACACT
	GACAATGGCT	CCAACTTCAC	TGGGGCCACA	GTGAGGGCTG	CCTGCTGGTG	GGCTGGCATC
	AAGCAGGAGT	TTGGCATCCC	CTACAACCCC	CAGTCCCAGG	GGGTGGTGGA	GTCCATGAAC
35	AAGGAGCTGA	AGAAGATCAT	TGGGCAGGTG	AGGGACCAGG	CTGAGCACCI	GAAGACAGCT
	GTGCAGATGG	CTGTGTTCAT	CCACAACTTC	AAGAGGAAGG	GGGGCATCGG	GGGCTACTCC

GCTGGGGAGA GGATTGTGGA CATCATTGCC ACAGACATCC AGACCAAGGA GCTCCAGAAG
CAGATCACCA AGATCCAGAA CTTCAGGGTG TACTACAGGG ACTCCAGGAA CCCCCTGTGG
AAGGGCCCTG CCAAGCTGCT GTGGAAGGGG GAGGGGGCTG TGGTGATCCA GGACAACTCT
GACATCAAGG TGGTGCCCAG GAGGAAGGCC AAGATCATCA GGGACTATGG CAAGCAGATG
GCTGGGGATG ACTGTGTGGC CTCCAGGCAG GATGAGGACT AAAGCCCGGG CAGATCT (SEQ
ID NO:1).

The open reading frame of the wild type pol construct disclosed as SEQ ID NO:1 contains 850 amino acids, disclosed herein as SEQ ID NO:2, as follows: Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys 10 Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Asp 15 Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Asp Asp Leu Tyr Val Gly 20 Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val 25 Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys 30 Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu 35 Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Asp Gly Ala Ala

Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Glu Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Asp Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro 10 Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Asp Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Asp Asn Gly Ser Asn Phe Thr Gly Ala Thr Val 15 Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Glu Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly 20 Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp 25 Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:2).

The present invention especially relates to an adenoviral vector vaccine which comprises a codon optimized HIV-1 DNA pol construct wherein, in addition to deletion of the portion of the wild type sequence encoding the protease activity, a combination of active site residue mutations are introduced which are deleterious to HIV-1 pol (RT-RH-IN) activity of the expressed protein. Therefore, the present invention preferably relates to an adenoviral HIV-1 DNA pol-based vaccine wherein the construct is devoid of DNA sequences encoding any PR activity, as well as containing a mutation(s) which at least partially, and preferably substantially, abolishes RT, RNase and/or IN activity. One type of HIV-1 pol mutant which is part and parcel of an adenoviral vector vaccine may include but is not limited to a mutated

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DNA molecule comprising at least one nucleotide substitution which results in a point mutation which effectively alters an active site within the RT, RNase and/or IN regions of the expressed protein, resulting in at least substantially decreased enzymatic activity for the RT, RNase H and/or IN functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a HIV-1 DNA pol construct contains a mutation or mutations within the Pol coding region which effectively abolishes RT, RNase H and IN activity. An especially preferable HIV-1 DNA pol construct in a DNA molecule which contains at least one point mutation which alters the active site of the RT, RNase H and IN domains of Pol, such that each activity is at least substantially abolished. Such a HIV-1 Pol mutant will most likely comprise at least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. To this end, an especially preferred HIV-1 DNA pol construct is exemplified herein and contains nine codon substitution mutations which results in an inactivated Pol protein (IA Pol: SEQ ID NO:4, Figure 17A-C) which has no PR, RT, RNase or IN activity, wherein three such point mutations reside within each of the RT, RNase and IN catalytic domains. Therefore, an especially preferred exemplification is an adenoviral vaccine which comprises, in an appropriate fashion, a DNA molecule which encodes IA-pol, which contains all nine mutations as shown below in Table 1. An additional preferred amino acid residue for substitution is Asp551, localized within the RNase domain of Pol. Any combination of the mutations disclosed herein may suitable and therefore may be utilized as an IA-Pol-based vaccine of the present invention. While addition and deletion mutations are contemplated and within the scope of the invention, the preferred mutation is a point mutation resulting in a substitution of the wild type amino acid with an alternative amino acid residue.

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•	wt aa	aa residue	mutant aa	enzyme function
	Asp	112	Ala	RT
	Asp	187	Ala	RT
30	Asp	188	Ala	RT
	Asp .	445	. Ala	. RNase H
	Glu	480	Ala	RNase H
	Asp	500	Ala	RNase H
	Asp	626	Ala	IN
35	Asp	678	Ala	IN
	Glu	714	Ala	IN

It is preferred that point mutations be incorporated into the IApol mutant adenoviral vaccines of the present invention so as to lessen the possibility of altering epitopes in and around the active site(s) of HIV-1 Pol.

To this end, SEQ ID NO:3 discloses the nucleotide sequence which codes for a codon optimized pol in addition to the nine mutations shown in Table 1, disclosed as follows, and referred to herein as "IApol":

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AGATCTACCA TGGCCCCCAT CTCCCCCATT GAGACTGTGC CTGTGAAGCT GAAGCCTGGC ATGGATGGCC CCAAGGTGAA GCAGTGGCCC CTGACTGAGG AGAAGATCAA GGCCCTGGTG GAAATCTGCA CTGAGATGGA GAAGGAGGGC AAAATCTCCA AGATTGGCCC CGAGAACCCC TACAACACCC CTGTGTTTGC CATCAAGAAG AAGGACTCCA CCAAGTGGAG GAAGCTGGTG GACTTCAGGG AGCTGAACAA GAGGACCCAG GACTTCTGGG AGGTGCAGCT GGGCATCCCC CACCCCGCTG GCCTGAAGAA GAAGAAGTCT GTGACTGTGC TGGCTGTGGG GGATGCCTAC TTCTCTGTGC CCCTGGATGA GGACTTCAGG AAGTACACTG CCTTCACCAT CCCCTCCATC AACAATGAGA CCCCTGGCAT CAGGTACCAG TACAATGTGC TGCCCCAGGG CTGGAAGGGC TCCCCTGCCA TCTTCCAGTC CTCCATGACC AAGATCCTGG AGCCCTTCAG GAAGCAGAAC CCTGACATTG TGATCTACCA GTACATGGCT GCCCTGTATG TGGGCTCTGA CCTGGAGATT GGGCAGCACA GGACCAAGAT TGAGGAGCTG AGGCAGCACC TGCTGAGGTG GGGCCTGACC ACCCCTGACA AGAAGCACCA GAAGGAGCCC CCCTTCCTGT GGATGGGCTA TGAGCTGCAC CCCGACAAGT GGACTGTGCA GCCCATTGTG CTGCCTGAGA AGGACTCCTG GACTGTGAAT GACATCCAGA AGCTGGTGGG CAAGCTGAAC TGGGCCTCCC AAATCTACCC TGGCATCAAG GTGAGGCAGC TGTGCAAGCT GCTGAGGGGC ACCAAGGCCC TGACTGAGGT GATCCCCCTG ACTGAGGAGG CTGAGCTGGA GCTGGCTGAG AACAGGGAGA TCCTGAAGGA GCCTGTGCAT GGGGTGTACT ATGACCCCTC CAAGGACCTG ATTGCTGAGA TCCAGAAGCA GGGCCAGGGC CAGTGGACCT ACCAAATCTA CCAGGAGCCC TTCAAGAACC TGAAGACTGG CAAGTATGCC AGGATGAGGG GGGCCCACAC CAATGATGTG AAGCAGCTGA CTGAGGCTGT GCAGAAGATC ACCACTGAGT CCATTGTGAT CTGGGGCAAG ACCCCCAAGT TCAAGCTGCC CATCCAGAAG GAGACCTGGG AGACCTGGTG GACTGAGTAC TGGCAGGCCA CCTGGATCCC TGAGTGGGAG TTTGTGAACA CCCCCCCCT GGTGAAGCTG TGGTACCAGC TGGAGAAGGA GCCCATTGTG GGGGCTGAGA CCTTCTATGT GGCTGGGGCT GCCAACAGGG AGACCAAGCT GGGCAAGGCT GGCTATGTGA CCAACAGGGG CAGGCAGAAG GTGGTGACCC TGACTGACAC CACCAACCAG AAGACTGCCC TCCAGGCCAT CTACCTGGCC CTCCAGGACT CTGGCCTGGA GGTGAACATT GTGACTGCCT CCCAGTATGC CCTGGGCATC ATCCAGGCCC AGCCTGATCA GTCTGAGTCT GTGCCTGCCC ACAAGGGCAT TGGGGGCAAT GAGCAGGTGG ACAAGCTGGT GTCTGCTGGC ATCAGGAAGG TGCTGTTCCT GGATGGCATT GACAAGGCCC AGGATGAGCA TGAGAAGTAC CACTCCAACT GGAGGGCTAT GGCCTCTGAC TTCAACCTGC CCCCTGTGGT GGCTAAGGAG

PCT/US01/28861 WO 02/022080

ATTGTGGCCT CCTGTGACAA GTGCCAGCTG AAGGGGGAGG CCATGCATGG GCAGGTGGAC TGCTCCCCTG GCATCTGGCA GCTGGCCTGC ACCCACCTGG AGGGCAAGGT GATCCTGGTG GCTGTGCATG TGGCCTCCGG CTACATTGAG GCTGAGGTGA TCCCTGCTGA GACAGGCCAG GAGACTGCCT ACTTCCTGCT GAAGCTGGCT GGCAGGTGGC CTGTGAAGAC CATCCACACT GCCAATGGCT CCAACTTCAC TGGGGCCACA GTGAGGGCTG CCTGCTGGTG GGCTGGCATC AAGCAGGAGT TTGGCATCCC CTACAACCCC CAGTCCCAGG GGGTGGTGGC CTCCATGAAC AAGGAGCTGA AGAAGATCAT TGGGCAGGTG AGGGACCAGG CTGAGCACCT GAAGACAGCT GTGCAGATGG CTGTGTTCAT CCACAACTTC AAGAGGAAGG GGGGCATCGG GGGCTACTCC GCTGGGGAGA GGATTGTGGA CATCATTGCC ACAGACATCC AGACCAAGGA GCTCCAGAAG CAGATCACCA AGATCCAGAA CTTCAGGGTG TACTACAGGG ACTCCAGGAA CCCCCTGTGG AAGGGCCCTG CCAAGCTGCT GTGGAAGGGG GAGGGGGCTG TGGTGATCCA GGACAACTCT GACATCAAGG TGGTGCCCAG GAGGAAGGCC AAGATCATCA GGGACTATGG CAAGCAGATG GCTGGGGATG ACTGTGTGGC CTCCAGGCAG GATGAGGACT AAAGCCCGGG CAGATCT (SEQ ID NO:3).

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In order to produce the IA-pol-based adenoviral vaccines of the present invention, inactivation of the enzymatic functions was achieved by replacing a total of nine active site residues from the enzyme subunits with alanine side-chains. As shown in Table 1, all residues that comprise the catalytic triad of the polymerase, namely Asp112, Asp187, and Asp188, were substituted with alanine (Ala) residues (Larder, et al., Nature 1987, 327: 716-717; Larder, et al., 1989, Proc. Natl. Acad. Sci. 20 1989, 86: 4803-4807). Three additional mutations were introduced at Asp445, Glu480 and Asp500 to abolish RNase H activity (Asp551 was left unchanged in this IA Pol construct), with each residue being substituted for an Ala residue, respectively (Davies, et al., 1991, Science 252:, 88-95; Schatz, et al., 1989, FEBS Lett. 257: 311-314; Mizrahi, et al., 1990, Nucl. Acids. Res. 18: pp. 5359-5353). HIV pol integrase 25 function was abolished through three mutations at Asp626, Asp678 and Glu714. Again, each of these residues has been substituted with an Ala residue (Wiskerchen, et al., 1995, J. Virol. 69: 376-386; Leavitt, et al., 1993, J. Biol. Chem. 268: 2113-2119). Amino acid residue Pro3 of SEQ ID NO:4 marks the start of the RT gene. The complete amino acid sequence of IA-Pol is disclosed herein as SEQ ID NO:4 and 30 Figure 17A-C, as follows:

Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg

Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Ser Val Thr Val Leu Ala Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys 10 Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr 15 Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile 20 Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly 25 Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys 30 . Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly

Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly Gly Gly Gly Gly Gly Gly Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asp Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:4).

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As noted above, it will be understood that any combination of the mutations disclosed above may be suitable and therefore be utilized as an IA-pol-based adenoviral HIV vaccine of the present invention, either when administered alone or in a combined modality regime and/or a prime-boost regimen. For example, it may be possible to mutate only 2 of the 3 residues within the respective reverse transcriptase, RNase-H, and integrase coding regions while still abolishing these enzymatic activities. However, the IA-pol construct described above and disclosed as SEQ ID NO:3, as well as the expressed protein (SEQ ID NO:4;) is preferred. It is also preferred that at least one mutation be present in each of the three catalytic domains.

Another aspect of this portion of the invention are codon optimized HIV-1 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal peptide such as from tPA (tissue-type plasminogen activator) or by a leader peptide such as is found in highly expressed mammalian proteins such as immunoglobulin leader peptides. Any functional leader peptide may be tested for efficacy. However, a preferred embodiment of the present invention, as with HIV-1 Nef constructs shown herein, is to provide for a HIV-1 Pol mutant adenoviral vaccine construction wherein the pol coding region or a portion thereof is operatively linked to a leader peptide, preferably a leader peptide from human tPA. In other words, a codon optimized HIV-1 Pol mutant such as IA-Pol (SEQ ID NO:4) may also comprise a leader peptide at the amino terminal portion of the protein, which may effect cellular trafficking and hence, immunogenicity of the expressed protein within the host cell. As noted in Figure 16A-B, a DNA vector which may be utilized to practice the present invention may be modified by known recombinant DNA methodology to contain a leader signal

peptide of interest, such that downstream cloning of the modified HIV-1 protein of interest results in a nucleotide sequence which encodes a modified HIV-1 tPA/Pol protein. In the alternative, as noted above, insertion of a nucleotide sequence which encodes a leader peptide may be inserted into a DNA vector housing the open reading frame for the Pol protein of interest. Regardless of the cloning strategy, the end result is a polynucleotide vaccine which comprises vector components for effective gene expression in conjunction with nucleotide sequences which encode a modified HIV-1 Pol protein of interest, including but not limited to a HIV-1 Pol protein which contains a leader peptide. The amino acid sequence of the human tPA leader utilized herein is as follows: MDAMKRGLCCVLLLCGAVFVSPSEISS (SEQ ID NO:17). Therefore, another aspect of the present invention is to generate HIV-1 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal peptide such as from tPA. To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity is deleted and a human tPA leader sequence is fused to the 5' end of the coding region. A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame disclosed herein as SEQ ID NO:6.

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To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity is deleted and a human tPA leader sequence is fused to the 5' end of the coding region (herein, "tPA-wt-pol"). A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame being contained from an initiating Met residue at nucleotides 8-10 to a termination codon from nucleotides 2633-2635. SEQ ID NO:5 is as follows:

GATCACCATG GATGCAATGA AGAGAGGGCT CTGCTGTGT CTGCTGTT GTGGAGCAGT
CTTCGTTTCG CCCAGCGAGA TCTCCGCCCC CATCTCCCC ATTGAGACTG TGCCTGTGAA
GCTGAAGCCT GGCATGGATG GCCCCAAGGT GAAGCAGTGG CCCCTGACTG AGGAGAAGAT
CAAGGCCCTG GTGGAAATCT GCACTGAGAT GGAGAAGGAG GGCAAAATCT CCAAGATTGG
CCCCGAGAAC CCCTACAACA CCCCTGTGTT TGCCATCAAG AAGAAGGACT CCACCAAGTG
GAGGAAGCTG GTGGACTTCA GGGAGCTGAA CAAGAGGACC CAGGACTTCT GGGAGGTGCA
GCTGGGCATC CCCCACCCCG CTGGCCTGAA GAAGAAGAAG TCTGTGACTG TGCTGGATGT
GGGGGATGCC TACTTCTCTG TGCCCCTGGA TGAGGACTTC AGGAAGTACA CTGCCTTCAC
CATCCCCTCC ATCAACAATG AGACCCCTGG CATCAGGTAC CAGTACAATG TGCTGCCCCA
GGGCTGGAAG GGCTCCCCTG CCATCTTCCA GTCCTCCATG ACCAAGATCC TGGAGCCCTT
CAGGAAGCAG AACCCTGACA TTGTGATCTA CCAGTACATG GATGACCTGT ATGTGGGCTC
TGACCTGGAG ATTGGGCAGC ACAGGACCAA GATTGAGGAG CTGAGGCCAGC ACCTGCTGAG

GTGGGGCCTG ACCACCCTG ACAAGAAGCA CCAGAAGGAG CCCCCCTTCC TGTGGATGGG CTATGAGCTG CACCCCGACA AGTGGACTGT GCAGCCCATT GTGCTGCCTG AGAAGGACTC CTGGACTGTG AATGACATCC AGAAGCTGGT GGGCAAGCTG AACTGGGCCT CCCAAATCTA CCCTGGCATC AAGGTGAGGC AGCTGTGCAA GCTGCTGAGG GGCACCAAGG CCCTGACTGA GGTGATCCCC CTGACTGAGG AGGCTGAGCT GGAGCTGGCT GAGAACAGGG AGATCCTGAA GGAGCCTGTG CATGGGGTGT ACTATGACCC CTCCAAGGAC CTGATTGCTG AGATCCAGAA GCAGGGCCAG GGCCAGTGGA CCTACCAAAT CTACCAGGAG CCCTTCAAGA ACCTGAAGAC TGGCAAGTAT GCCAGGATGA GGGGGGCCCA CACCAATGAT GTGAAGCAGC TGACTGAGGC TGTGCAGAAG ATCACCACTG AGTCCATTGT GATCTGGGGC AAGACCCCCA AGTTCAAGCT GCCCATCCAG AAGGAGACCT GGGAGACCTG GTGGACTGAG TACTGGCAGG CCACCTGGAT CCCTGAGTGG GAGTTTGTGA ACACCCCCC CCTGGTGAAG CTGTGGTACC AGCTGGAGAA GGAGCCCATT GTGGGGGCTG AGACCTTCTA TGTGGATGGG GCTGCCAACA GGGAGACCAA CCTGGCCAAG GCTGGCTATG TGACCAACAG GGGCAGGCAG AAGGTGGTGA CCCTGACTGA CACCACCAAC CAGAAGACTG AGCTCCAGGC CATCTACCTG GCCCTCCAGG ACTCTGGCCT 15 GGAGGTGAAC ATTGTGACTG ACTCCCAGTA TGCCCTGGGC ATCATCCAGG CCCAGCCTGA TCAGTCTGAG TCTGAGCTGG TGAACCAGAT CATTGAGCAG CTGATCAAGA AGGAGAAGGT GTACCTGGCC TGGGTGCCTG CCCACAAGGG CATTGGGGGC AATGAGCAGG TGGACAAGCT GGTGTCTGCT GGCATCAGGA AGGTGCTGTT CCTGGATGGC ATTGACAAGG CCCAGGATGA GCATGAGAAG TACCACTCCA ACTGGAGGGC TATGGCCTCT GACTTCAACC TGCCCCCTGT GGTGGCTAAG GAGATTGTGG CCTCCTGTGA CAAGTGCCAG CTGAAGGGGG AGGCCATGCA 20 TGGGCAGGTG GACTGCTCCC CTGGCATCTG GCAGCTGGAC TGCACCCACC TGGAGGGCAA GGTGATCCTG GTGGCTGTGC ATGTGGCCTC CGGCTACATT GAGGCTGAGG TGATCCCTGC TGAGACAGGC CAGGAGACTG CCTACTTCCT GCTGAAGCTG GCTGGCAGGT GGCCTGTGAA GACCATCCAC ACTGACAATG GCTCCAACTT CACTGGGGCC ACAGTGAGGG CTGCCTGCTG GTGGGCTGGC ATCAAGCAGG AGTTTGGCAT CCCCTACAAC CCCCAGTCCC AGGGGGTGGT 25 GGAGTCCATG AACAAGGAGC TGAAGAAGAT CATTGGGCAG GTGAGGGACC AGGCTGAGCA CCTGAAGACA GCTGTGCAGA TGGCTGTTT CATCCACAAC TTCAAGAGGA AGGGGGGCAT CGGGGGCTAC TCCGCTGGGG AGAGGATTGT GGACATCATT GCCACAGACA TCCAGACCAA GGAGCTCCAG AAGCAGATCA CCAAGATCCA GAACTTCAGG GTGTACTACA GGGACTCCAG GAACCCCTG TGGAAGGGCC CTGCCAAGCT GCTGTGGAAG GGGGAGGGGG CTGTGGTGAT 30 CCAGGACAAC TCTGACATCA AGGTGGTGCC CAGGAGGAAG GCCAAGATCA TCAGGGACTA TGGCAAGCAG ATGGCTGGGG ATGACTGTGT GGCCTCCAGG CAGGATGAGG ACTAAAGCCC GGGCAGATCT (SEQ ID NO:5).

The open reading frame of the wild type tPA-pol construct disclosed as SEQ ID NO:5 contains 875 amino acids, disclosed herein as SEQ ID NO:6, as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Cys Gly

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Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Ser Val Thr Val Leu Asp Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro 10 Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Asp Asp Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly 15 Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile 20 Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln 25 Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Asp Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly 30 Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr.Glu Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Asp Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu 35 Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile

Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Asp Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu 5 Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Asp Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Glu Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val 10 Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp 15 Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:6).

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The present invention also relates to a codon optimized HIV-1 Pol mutant contained within a recombinant adenoviral vector such as IA-Pol (SEQ ID NO:4) which comprises a leader peptide at the amino terminal portion of the protein, which may effect cellular trafficking and hence, immunogenicity of the expressed protein within the host cell. Any such adenoviral-based HIV-1 DNA pol mutant disclosed in the above paragraphs is suitable for fusion downstream of a leader peptide, such as a leader peptide including but not limited to the human tPA leader sequence. Therefore, any such leader peptide-based HIV-1 pol mutant construct may include but is not limited to a mutated DNA molecule which effectively alters the catalytic activity of the RT, RNase and/or IN region of the expressed protein, resulting in at least substantially decreased enzymatic activity one or more of the RT, RNase H and/or IN functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a leader peptide/HIV-1 DNA pol construct contains a mutation or mutations within the Pol coding region which effectively abolishes RT, RNase H and IN activity. An especially preferable HIV-1 DNA pol construct is a DNA molecule which contains at least one point mutation which alters the active site and catalytic activity within the RT, RNase H and IN domains of Pol, such that each activity is at least substantially abolished, and preferably totally abolished. Such a HIV-1 Pol mutant will most likely

comprise at least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. An especially preferred embodiment of this portion of the invention relates to a human tPA leader fused to the IA-Pol protein comprising the nine mutations shown in Table 1. The DNA molecule is disclosed herein as SEQ ID NO:7 and the expressed tPA-IA Pol protein comprises a fusion 5 junction as shown in Figure 18. The complete amino acid sequence of the expressed protein is set forth in SEQ ID NO:8. To this end, SEQ ID NO:7 discloses the nucleotide sequence which codes for a human tPA leader fused to the IA Pol protein comprising the nine mutations shown in Table 1 (herein, "tPA-opt-IApol"). The open reading frame begins with the initiating Met (nucleotides 8-10) and terminates with a "TAA" codon at nucleotides 2633-2635. The nucleotide sequence encoding tPA-IAPol is also disclosed as follows: GATCACCATG GATGCAATGA AGAGAGGGCT CTGCTGTGTG CTGCTGCTGT GTGGAGCAGT CTTCGTTTCG CCCAGCGAGA TCTCCGCCCC CATCTCCCCC ATTGAGACTG TGCCTGTGAA GCTGAAGCCT GGCATGGATG GCCCCAAGGT GAAGCAGTGG CCCCTGACTG AGGAGAAGAT CAAGGCCCTG GTGGAAATCT GCACTGAGAT GGAGAAGGAG GGCAAAATCT CCAAGATTGG CCCCGAGAAC CCCTACAACA CCCCTGTGTT TGCCATCAAG AAGAAGGACT CCACCAAGTG GAGGAAGCTG GTGGACTTCA GGGAGCTGAA CAAGAGGACC CAGGACTTCT GGGAGGTGCA GCTGGGCATC CCCCACCCCG CTGGCCTGAA GAAGAAGAAG TCTGTGACTG TGCTGGCTGT GGGGGATGCC TACTTCTCTG TGCCCCTGGA TGAGGACTTC AGGAAGTACA CTGCCTTCAC CATCCCCTCC ATCAACAATG AGACCCCTGG CATCAGGTAC CAGTACAATG TGCTGCCCCA GGGCTGGAAG GGCTCCCCTG CCATCTTCCA GTCCTCCATG ACCAAGATCC TGGAGCCCTT CAGGAAGCAG AACCCTGACA TTGTGATCTA CCAGTACATG GCTGCCCTGT ATGTGGGCTC TGACCTGGAG ATTGGGCAGC ACAGGACCAA GATTGAGGAG CTGAGGCAGC ACCTGCTGAG GTGGGGCCTG ACCACCCCTG ACAAGAAGCA CCAGAAGGAG CCCCCCTTCC TGTGGATGGG CTATGAGCTG CACCCCGACA AGTGGACTGT GCAGCCCATT GTGCTGCCTG AGAAGGACTC CTGGACTGTG AATGACATCC AGAAGCTGGT GGGCAAGCTG AACTGGGCCT CCCAAATCTA CCCTGGCATC AAGGTGAGGC AGCTGTGCAA GCTGCTGAGG GGCACCAAGG CCCTGACTGA GGTGATCCCC CTGACTGAGG AGGCTGAGCT GGAGCTGGCT GAGAACAGGG AGATCCTGAA GGAGCCTGTG CATGGGGTGT ACTATGACCC CTCCAAGGAC CTGATTGCTG AGATCCAGAA -GCAGGGCCAG GGCCAGTGGA CCTACCAAAT CTACCAGGAG CCCTTCAAGA ACCTGAAGAC TGGCAAGTAT GCCAGGATGA GGGGGGCCCA CACCAATGAT GTGAAGCAGC TGACTGAGGC TGTGCAGAAG ATCACCACTG AGTCCATTGT GATCTGGGGC AAGACCCCCA AGTTCAAGCT GCCCATCCAG AAGGAGACCT GGGAGACCTG GTGGACTGAG TACTGGCAGG CCACCTGGAT CCCTGAGTGG GAGTTTGTGA ACACCCCCCC CCTGGTGAAG CTGTGGTACC AGCTGGAGAA GGAGCCCATT GTGGGGGCTG AGACCTTCTA TGTGGCTGGG GCTGCCAACA GGGAGACCAA

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GCTGGGCAAG GCTGGCTATG TGACCAACAG GGGCAGGCAG AAGGTGGTGA CCCTGACTGA CACCACCAAC CAGAAGACTG CCCTCCAGGC CATCTACCTG GCCCTCCAGG ACTCTGGCCT GGAGGTGAAC ATTGTGACTG CCTCCCAGTA TGCCCTGGGC ATCATCCAGG CCCAGCCTGA TCAGTCTGAG TCTGAGCTGG TGAACCAGAT CATTGAGCAG CTGATCAAGA AGGAGAAGGT GTACCTGGCC TGGGTGCCTG CCCACAAGGG CATTGGGGGC AATGAGCAGG TGGACAAGCT GGTGTCTGCT GGCATCAGGA AGGTGCTGTT CCTGGATGGC ATTGACAAGG CCCAGGATGA GCATGAGAAG TACCACTCCA ACTGGAGGGC TATGGCCTCT GACTTCAACC TGCCCCTGT GGTGGCTAAG GAGATTGTGG CCTCCTGTGA CAAGTGCCAG CTGAAGGGGG AGGCCATGCA TGGGCAGGTG GACTGCTCCC CTGGCATCTG GCAGCTGGCC TGCACCCACC TGGAGGGCAA GGTGATCCTG GTGGCTGTGC ATGTGGCCTC CGGCTACATT GAGGCTGAGG TGATCCCTGC TGAGACAGGC CAGGAGACTG CCTACTTCCT GCTGAAGCTG GCTGGCAGGT GGCCTGTGAA GACCATCCAC ACTGCCAATG GCTCCAACTT CACTGGGGCC ACAGTGAGGG CTGCCTGCTG GTGGGCTGGC ATCAAGCAGG AGTTTGGCAT CCCCTACAAC CCCCAGTCCC AGGGGGTGGT GGCCTCCATG AACAAGGAGC TGAAGAAGAT CATTGGGCAG GTGAGGGACC AGGCTGAGCA CCTGAAGACA GCTGTGCAGA TGGCTGTGTT CATCCACAAC TTCAAGAGGA AGGGGGGCAT CGGGGGCTAC TCCGCTGGGG AGAGGATTGT GGACATCATT GCCACAGACA TCCAGACCAA GGAGCTCCAG AAGCAGATCA CCAAGATCCA GAACTTCAGG GTGTACTACA GGGACTCCAG GAACCCCTG TGGAAGGGCC CTGCCAAGCT GCTGTGGAAG GGGGAGGGGG CTGTGGTGAT CCAGGACAAC TCTGACATCA AGGTGGTGCC CAGGAGGAAG GCCAAGATCA TCAGGGACTA TGGCAAGCAG ATGGCTGGGG ATGACTGTGT GGCCTCCAGG CAGGATGAGG ACTAAAGCCC 20 GGGCAGATCT (SEQ ID NO:7).

The open reading frame of the tPA-IA-pol construct disclosed as SEQ ID NO:7 contains 875 amino acids, disclosed herein as tPA-IA-Pol and SEQ ID NO:8, as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser Pro Ile
Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val
Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile
Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu
30 Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr
Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln
Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys
Lys Lys Lys Ser Val Thr Val Leu Ala Val Gly Asp Ala Tyr Phe Ser
Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro
35 Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu
Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr

Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile 10 Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr 15 Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu 20 Thr Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp 25 Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu 30 Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe. Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly 35 Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe

Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:8).

EXAMPLE 18

CODON OPTIMIZED HIV-1 NEF AND CODON OPTIMIZED HIV-1 NEF MODIFICATIONS

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Codon optimized version of HIV-1 Nef and HIV-1 Nef modifications are essentially as described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference. As disclosed within the above-mentioned documents, particular embodiments of codon optimized Nef and Nef modifications relate to a DNA molecule encoding HIV-1 Nef from the HIV-1 ifrl isolate wherein the codons are optimized for expression in a mammalian system such as a human. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:9, while the expressed open reading frame is disclosed herein as SEQ ID NO:10. Another embodiment of Nef-based coding regions for use in the adenoviral vectors of the present invention comprise a codon optimized DNA molecule encoding a protein containing the human plasminogen activator (tpa) leader peptide fused with the NH₂-terminus of the HIV-1 Nef polypeptide. The DNA molecule which encodes this protein is disclosed herein as SEO ID NO:11, while the expressed open reading frame is disclosed herein as SEQ ID NO:12. Another modified Nef optimized coding region relates to a DNA molecule encoding optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175, herein described as opt nef (G2A, LLAA). The DNA molecule which encodes this protein is disclosed herein as SEO ID NO:13, while the expressed open reading frame is disclosed herein as SEQ ID NO:14. An additional embodiment relates to a DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA), comprises an open reading frame which

encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HTV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175, herein referred to as opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, while the expressed open reading frame is disclosed herein as SEO ID NO:16.

As disclosed in the above-identified documents (U.S. Application Serial No. 09/738,782 and PCT International Application PCT/US00/34162) and reiterated herein, the following nef-based nucleotide and amino acid sequences which comprise the respective open reading frame are as follows:

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The nucleotide sequence of the codon optimized version of HIV-1 irfl nef gene is disclosed herein as SEQ ID NO:9, as shown herein:

GATCTGCCAC CATGGGCGGC AAGTGGTCCA AGAGGTCCGT GCCCGGCTGG TCCACCGTGA GGGAGAGGAT GAGGAGGCC GAGCCCGCCG CCGACAGGT GAGGAGGACC GAGCCCGCCG CCGTGGCGT GGGCGCCGTG TCCAGGGACC TGGAGAAGCA CGGCGCCATC ACCTCCTCCA ACACCGCCGC CACCAACGCC GACTGCGCCT GGCTGGAGGC CCAGGAGGAC GAGGAGGTGG GCTTCCCCGT GAGGCCCCAG GTGCCCCTGA GGCCCATGAC CTACAAGGGC GCCGTGGACC TGTCCCACTT CCTGAAGGAG AAGGGCGGCC TGGAGGGCCT GATCCACTCC CAGAAGAGGC AGGACATCCT GGACCTGTGG GTGTACCACA CCCAGGGCTA CTTCCCCGAC TGGCAGAACT ACACCCCGG CCCCGGCATC AGGTTCCCCC TGACCTTCGG CTGGTGCTTC AAGCTGGTGC CCGTGGAGCC CGAGAAGGTG GAGGAGGCCA ACGAGGGCGA GAACAACTGC CTGCTGCACC CCATGTCCCA GCACGGCATC GAGGACCCCG AGAAGGAGGT GCTGGAGTGG AGGTTCGACT CCAAGCTGGC CTTCCACCAC GTGGCCAGGG AGCTGCACCC CGAGTACTAC AAGGACTGCT AAAGCCCGGG C (SEQ ID NO:9).

Preferred codon usage is as follows: Met (ATG), Gly (GGC), Lys (AAG), Trp (TGG), Ser (TCC), Arg (AGG), Val (GTG), Pro (CCC), Thr (ACC), Glu (GAG); Leu (CTG), His (CAC), Ile (ATC), Asn (AAC), Cys (TGC), Ala (GCC), Gln (CAG), Phe (TTC) and Tyr (TAC). For an additional discussion relating to mammalian (human) codon optimization, see WO 97/31115 (PCT/US97/02294), which is hereby incorporated by reference. See also Figure 19A-B for a comparion of wild type vs. codon optimized nucleotides comprising the open reading frame of HIV-Nef.

The open reading frame for SEQ ID NO:9 above comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662. The open reading frame of SEQ ID NO:9 provides for a 216 amino acid HIV-1 Nef protein expressed through utilization of a codon optimized DNA vaccine vector. The 216 amino acid HIV-1 Nef (ifrl) protein is disclosed herein as SEQ ID 35 NO:10, and as follows:

Met Gly Gly Lys Trp Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val

Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Leu Leu His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:10).

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HIV-1 Nef is a 216 amino acid cytosolic protein which associates with the inner surface of the host cell plasma membrane through myristylation of Gly-2 (Franchini et al., 1986, Virology 155: 593-599). While not all possible Nef functions have been elucidated, it has become clear that correct trafficking of Nef to the inner plasma membrane promotes viral replication by altering the host intracellular environment to facilitate the early phase of the HIV-1 life cycle and by increasing the infectivity of progeny viral particles. In one aspect of the invention regarding codon-optimized, protein-modified polypeptides, the nef-encoding region of the adenovirus vector of the present invention is modified to contain a nucleotide sequence which encodes a heterologous leader peptide such that the amino terminal region of the expressed protein will contain the leader peptide. The diversity of function that typifies eukaryotic cells depends upon the structural differentiation of their membrane boundaries. To generate and maintain these structures, proteins must be transported from their site of synthesis in the endoplasmic reticulum to predetermined destinations throughout the cell. This requires that the trafficking proteins display sorting signals that are recognized by the molecular machinery responsible for route selection located at the access points to the main trafficking pathways. Sorting decisions for most proteins need to be made only once as they traverse their biosynthetic pathways since their final destination, the cellular location at which they perform their function, becomes their permanent residence. Maintenance of intracellular integrity depends in part on the selective sorting and accurate transport of proteins to their correct destinations. Defined sequence motifs exist in proteins which can act as 'address labels'. A number of sorting signals have

been found associated with the cytoplasmic domains of membrane proteins. An effective induction of CTL responses often required sustained, high level endogenous expression of an antigen. As membrane-association via myristylation is an essential requirement for most of Nef's function, mutants lacking myristylation, by glycine-to-alanine change, change of the dileucine motif and/or by substitution with a tpa leader sequence as described herein, will be functionally defective, and therefore will have improved safety profile compared to wild-type Nef for use as an HIV-1 vaccine component.

In another embodiment of this portion of the invention, either the DNA vector or the HIV-1 nef nucleotide sequence is modified to include the human tissue-specific plasminogen activator (tPA) leader. As shown in Figure 16A-B, a DNA vector may be modified by known recombinant DNA methodology to contain a leader signal peptide of interest, such that downstream cloning of the modified HIV-1 protein of interest results in a nucleotide sequence which encodes a modified HIV-1 tPA/Nef protein. In the alternative, as noted above, insertion of a nucleotide sequence which encodes a leader peptide may be inserted into a DNA vector housing the open reading frame for the Nef protein of interest. Regardless of the cloning strategy, the end result is a polynucleotide vaccine which comprises vector components for effective gene expression in conjunction with nucleotide sequences which encode a modified HIV-1 Nef protein of interest, including but not limited to a HIV-1 Nef protein which contains a leader peptide. The amino acid sequence of the human tPA leader utilized herein is as follows: MDAMKRGLCCVLLLCGAVFVSPSEISS (SEQ ID NO:17).

It has been shown that myristylation of Gly-2 in conjunction with a dileucine motif in the carboxy region of the protein is essential for Nef-induced down regulation of CD4 (Aiken et al., 1994, Cell 76: 853-864) via endocytosis. It has also been shown that Nef expression promotes down regulation of MHCI (Schwartz et al., 1996, Nature Medicine 2(3): 338-342) via endocytosis. The present invention relates in part to DNA vaccines which encode modified Nef proteins altered in trafficking and/or functional properties. The modifications introduced into the adenoviral vector HIV vaccines of the present invention include but are not limited to additions, deletions or substitutions to the nef open reading frame which results in the expression of a modified Nef protein which includes an amino terminal leader peptide, modification or deletion of the amino terminal myristylation site, and modification or deletion of the dileucine motif within the Nef protein and which alter function within the infected host cell. Therefore, a central theme of the DNA molecules and recombinant adenoviral HIV vaccines of the present invention is (1)

host administration and intracellular delivery of a codon optimized nef-based adenoviral HIV vaccine; (2) expression of a modified Nef protein which is immunogenic in terms of eliciting both CTL and Th responses; and, (3) inhibiting or at least altering known early viral functions of Nef which have been shown to promote HIV-1 replication and load within an infected host. Therefore, the nef coding region may be altered, resulting in a DNA vaccine which expresses a modified Nef protein wherein the amino terminal Gly-2 myristylation residue is either deleted or modified to express alternate amino acid residues. Also, the nef coding region may be altered so as to result in a DNA vaccine which expresses a modified Nef protein wherein the dileucine motif is either deleted or modified to express alternate amino acid residues. In addition, the adenoviral vector HIV vaccines of the present invention also relate to an isolated DNA molecule, regardless of codon usage, which expresses a wild type or modified Nef protein as described herein, including but not limited to modified Nef proteins which comprise a deletion or substitution of Gly 2, a deletion or substitution of Leu 174 and Leu 175 and/or inclusion of a leader sequence.

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Therefore, specific Nef-based constructs further include the following, as exemplification's and not limitations. For example, the present invention relates to an adenoviral vector vaccine which encodes modified forms of HIV-1, an open reading frame which encodes a Nef protein which comprises a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl) is referred to herein as opt tpanef. The nucleotide sequence comprising the open reading frame of opt tpanef is disclosed herein as SEQ ID NO:11, as shown below:

CATGGATGCA ATGAAGAGA GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT
TTCGCCCAGC GAGATCTCCT CCAAGAGGTC CGTGCCCGGC TGGTCCACCG TGAGGGAGAG
GATGAGGAGG GCCGAGCCCG CCGCCGACAG GGTGAGGAGG ACCGAGCCCG CCGCCGTGGG
CGTGGGCGCC GTGTCCAGGG ACCTGGAGAA GCACGGCGCC ATCACCTCCT CCAACACCGC
CGCCACCAAC GCCGACTGCG CCTGGCTGGA GGCCCAGGAG GACGAGGAGG TGGGCTTCCC
CGTGAGGCCC CAGGTGCCCC TGAGGCCCAT GACCTACAAG GGCGCCGTGG ACCTGTCCCA
CTTCCTGAAG GAGAAGGGCG GCCTGGAGGG CCTGATCCAC TCCCAGAAGA GGCAGGACAT
CCTGGACCTG TGGGTGTACC ACACCCAGGG CTACTTCCCC GACTGGCAGA ACTACACCCC
CGGCCCCGGC ATCAGGTTCC CCCTGACCTT CGGCTGGTGC TTCAAGCTGG TGCCCGTGGA
GCCCGAGAAG GTGGAGGAGG CCAACGAGGG CGAGAACAAC TGCCTGCTGC ACCCCATGTC
CCAGCACGGC ATCGAGGACC CCGAGAAGGA GGTCCTGGAG TGGAGGTTCG ACCCCATGTC
GGCCTTCCAC CACGTGGCCA GGGAGCTGCA CCCCGAGTAC TACAAGGACT GCTAAAGCC
(SEO ID NO:11).

The open reading frame for SEQ ID NO:11 comprises an initiating methionine

residue at nucleotides 2-4 and a "TAA" stop codon from nucleotides 713-715. The open reading frame of SEQ ID NO:3 provides for a 237 amino acid HIV-1 Nef protein which comprises a tPA leader sequence fused to amino acids 6-216 of HIV-1 Nef, including the dileucine motif at amino acid residues 174 and 175. This 237 amino acid tPA/Nef (jfrl) fusion protein is disclosed herein as SEQ ID NO:12, and is shown as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala 10 Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp 15 Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Leu Leu His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His 20 Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:12). Therefore, this exemplified Nef protein, Opt tPA-Nef, contains both a tPA leader sequence as well as deleting the myristylation site of Gly-2A DNA molecule encoding HIV-1 Nef from the HIV-1 ifrl isolate wherein the codons are optimized for expression in a mammalian system such as a human. 25

In another specific embodiment of the present invention, a DNA molecule is disclosed which encodes optimized HIV-1 Nef wherein the open reading frame of a recombinant adenoviral HIV vaccine encodes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175. This open reading frame is herein described as opt nef (G2A,LLAA) and is disclosed as SEQ ID NO:13, which comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662. The nucleotide sequence of this codon optimized version of HIV-1 jrfl nef gene with the above mentioned modifications is disclosed herein as SEQ ID NO:13, as follows:

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GATCTGCCAC CATGGCCGGC AAGTGGTCCA AGAGGTCCGT GCCCGGCTGG TCCACCGTGA
GGGAGAGGAT GAGGAGGGCC GAGCCCGCCG CCGACAGGGT GAGGAGGACC GAGCCCGCCG
CCGTGGGCGT GGGCGCCGTG TCCAGGGACC TGGAGAAGCA CGGCGCCATC ACCTCCTCCA
ACACCGCCGC CACCAACGCC GACTGCGCCT GGCTGGAGGC CCAGGAGGAC GAGGAGGTGG
GCTTCCCCGT GAGGCCCCAG GTGCCCCTGA GGCCCATGAC CTACAAGGGC GCCGTGGACC
TGTCCCACTT CCTGAAGGAG AAGGGCGGCC TGGAGGGCCT GATCCACTCC CAGAAGAGGC
AGGACATCCT GGACCTGTGG GTGTACCACA CCCAGGGCTA CTTCCCCGAC TGGCAGAACT
ACACCCCCGG CCCCGGCATC AGGTTCCCCC TGACCTTCGG CTGGTGCTTC AAGCTGGTGC
CCGTGGAGCC CGAGAAGGTG GAGGAGGCCA ACGAGGGGG GAACAACTGC GCCGCCCACC
CCATGTCCCA GCACGGCATC GAGGACCCCG AGAAGGAGGT GCTGGAGTGG AGGTTCGACT
CCAAGCTGGC CTTCCACCAC GTGGCCAGGG AGCTGCACCC CGAGTACTAC AAGGACTGCT
AAAGCCCGGG C (SEQ ID NO:13).

The open reading frame of SEQ ID NO:13 encodes Nef (G2A,LLAA), disclosed herein as SEQ ID NO:14, as follows:

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Met Ala Gly Lys Trp Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val 15 Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp 20 Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Ala Ala His 25 Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys Ser (SEQ ID NO:14).

An additional embodiment of the present invention relates to another DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA) comprises an open reading frame which encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175 (Ala-195 and Ala-196 in this tPA-based fusion protein). The nucleotide

sequence comprising the open reading frame of opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, as shown below:

CATGGATGCA ATGAAGAGAG GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT
TTCGCCCAGC GAGATCTCCT CCAAGAGGTC CGTGCCCGGC TGGTCCACCG TGAGGGAGAG
GATGAGGAGG GCCGAGCCCG CCGCCGACAG GGTGAGGAGG ACCGAGCCCG CCGCCGTGGG
CGTGGGCGCC GTGTCCAGGG ACCTGGAGAA GCACGGCGCC ATCACCTCCT CCAACACCGC
CGCCACCAAC GCCGACTGCG CCTGGCTGGA GGCCCAGGAG GACGAGGAGG TGGGCTTCCC
CGTGAGGCCC CAGGTGCCCC TGAGGCCCAT GACCTACAAG GGCGCCGTGG ACCTGTCCCA
CTTCCTGAAG GAGAAGGGCG GCCTGGAGGG CCTGATCCAC TCCCAGAAGA GGCAGGACAT
CCTGGACCTG TGGGTGTACC ACACCCAGGG CTACTTCCCC GACTGGCAGA ACTACACCCC
CGGCCCCGGC ATCAGGTTCC CCCTGACCTT CGGCTGGTGC TTCAAGCTGG TGCCCGTGGA
GCCCGAGAAG GTGGAGGAGG CCAACGAGGG CGAGAACAAC TGCGCCGCCC ACCCCATGTC
CCAGCACGGC ATCGAGGACC CCGAGAAGGA GGTGCTGGAG TGGAGGTTCG ACTCCAAGCT
GGCCTTCCAC CACGTGGCCA GGGAGCTGCA CCCCGAGTAC TACAAGGACT GCTAAAGCCC
(SEQ ID NO:15).

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The open reading frame of SEQ ID NO:7 encoding tPA-Nef (LLAA), disclosed herein as SEQ ID NO:16, is as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ser Lys Arg Ser Val Pro 20 Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn 30 Asn Cys Ala Ala His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:16). An adenoviral vector of the present invention may comprise a DNA sequence, regardless of codon usage, which expresses a wild type or modified Nef protein as 35 described herein, including but not limited to modified Nef proteins which comprise a deletion or substitution of Gly 2, a deletion of substitution of Leu 174 and Leu 175

and/or inclusion of a leader sequence. Therefore, partial or fully codon optimized DNA vaccine expression vector constructs are preferred since such constructs should result in increased host expression. However, it is within the scope of the present invention to utilize "non-codon optimized" versions of the constructs disclosed herein, especially modified versions of HIV Nef which are shown to promote a substantial cellular immune response subsequent to host administration.

Figure 20A-C show nucleotide sequences at junctions between nef coding sequence and plasmid backbone of nef expression vectors V1Jns/nef (Figure 20A), V1Jns/nef(G2A,LLAA) (Figure 20B), V1Jns/tpanef (Figure 20C) and V1Jns/tpanef(LLAA) (Figure 20C, also). 5' and 3' flanking sequences of codon optimized nef or codon optimized nef mutant genes are indicated by bold/italic letters; nef and nef mutant coding sequences are indicated by plain letters. Also indicated (as underlined) are the restriction endonuclease sites involved in construction of respective nef expression vectors. V1Jns/tpanef and V1Jns/tpanef(LLAA) have identical sequences at the junctions.

Figure 21 shows a schematic presentation of nef and nef derivatives. Amino acid residues involved in Nef derivatives are presented. Glycine 2 and Leucine 174 and 175 are the sites involved in myristylation and dileucine motif, respectively.

20 EXAMPLE 19

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MRKAd5Pol Construction and Virus Rescue

Steps performed in the construction of the vectors, including the pre-adenovirus plasmid - Key steps performed in the construction of the vectors, including the pre-adenovirus plasmid denoted MRKAd5pol, is depicted in Figure 22. Briefly, the adenoviral shuttle vector for the full-length inactivated HIV-1 pol gene is as follows. The vector MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) is a derivative of the shuttle vector used in the construction of the MRKAd5gag adenoviral pre-plasmid. The vector contains an expression cassette with the hCMV promoter (no intronA) and the bovine growth hormone polyadenylation signal. The expression unit has been inserted into the shuttle vector such that insertion of the gene of choice at a unique BgIII site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1-/E3+)Cla1 (or MRKpAdHVE3) pre-plasmid. The vector, similar to the original shuttle vector contains the Pac1 site, extension to the packaging signal region, and extension to the pIX gene. The synthetic full-length codon-optimized HIV-1 pol gene was isolated directly from the plasmid pV1Ins-HIV-pol-inact(opt). Digestion of this plasmid with BgI II releases the pol

gene intact (comprising a codon optimized IA pol sequence as disclosed in SEQ ID NO:3). The pol fragment was gel purified and ligated into the MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) shuttle vector at the BgIII site. The clones were checked for the correct orientation of the gene by using restriction enzymes DraIII/Not1. A positive clone was isolated and named MRKpdel+hCMVmin+FL-pol+bGHpA(s). The genetic structure of this plasmid was verified by PCR, restriction enzyme and DNA sequencing. The pre-adenovirus plasmid was constructed as follows. Shuttle plasmid MRKpdel+hCMVmin+FLpol+bGHpA(S) was digested with restriction enzymes Pac1 and Bst1107 I (or its isoschizomer, BstZ107 I) and then co-transformed into E. coli strain BJ5183 with linearized (Cla1 digested) adenoviral backbone plasmid, MRKpAd(E1-/E3+)Cla1. The resulting pre-plasmid originally named MRKpAd+hCMVmin+FLpol+bGHpA(S)E3+ is now referred to as "pMRKAd5pol". The genetic structure of the resulting pMRKAd5pol was verified by PCR, restriction enzyme and DNA sequence analysis. The vectors were transformed into competent E. coli XL-1 Blue for preparative production. The recovered plasmid was verified by restriction enzyme digestion and DNA sequence analysis, and by expression of the pol transgene in transient transfection cell culture. The complete nucleotide sequence of this pMRKAd5HIV-1pol adenoviral vector is shown in Figure 26 A-AO.

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Generation of research-grade recombinant adenovirus - The pre-adenovirus plasmid, pMRKAd5pol, was rescued as infectious virions in PER.C6® adherent monolayer cell culture. To rescue infectious virus, 12 μ g of pMRKAd5pol was digested with restriction enzyme Pacl (New England Biolabs) and 3.3 μ g was transfected per 6 cm dish of PER.C6® cells using the calcium phosphate co-precipitation technique (Cell Phect Transfection Kit, Amersham Pharmacia Biotech Inc.). Pacl digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into PER.C6® cells. Infected cells and media were harvested 6 -10 days post-transfection, after complete viral cytopathic effect (CPE) was observed. Infected cells and media were stored at \leq -60°C. This pol containing recombinant adenovirus is referred to herein as "MRKAd5pol". This recombinant adenovirus expresses an inactivated HIV-1 Pol protein as shown in SEQ ID NO:6.

EXAMPLE 20

MRKAd5Nef Construction and Virus Rescue

Construction of vector: shuttle plasmid and pre-adenovirus plasmid - Key steps performed in the construction of the vectors, including the pre-adenovirus

plasmid denoted MRKAd5nef, is depicted in Figure 23. Briefly, as shown in Example 19 above, the vector MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) is the shuttle vector used in the construction of the MRKAd5gag adenoviral pre-plasmid. It has been modified to contain the Pac1 site, extension to the packaging signal region, and extension to the pIX gene. It contains an expression cassette with the hCMV promoter (no intronA) and the bovine growth hormone polyadenylation signal. The expression unit has been inserted into the shuttle vector such that insertion of the gene of choice at a unique Bgl11 site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1-/E3+)Cla1 pre-plasmid. The synthetic full-length codon-optimized HIV-1 nef gene was isolated directly from the plasmid pV1Jns/nef (G2A,LLAA). Digestion of this plasmid with Bgl11 releases the pol gene intact, which comprises the nucleotide sequence as disclosed in SEQ ID NO:13. The nef fragment was gel purified and ligated into the

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MRKpdelE1+CMVmin+BGHpA(str.) shuttle vector at the Bgl11 site. The clones were checked for correction orientation of the gene by using restriction enzyme Scal. A positive clone was isolated and named MRKpdelE1hCMVminFL-nefBGHpA(s). The genetic structure of this plasmid was verified by PCR, restriction enzyme and DNA sequencing. The pre-adenovirus plasmid was constructed as follows. Shuttle plasmid MRKpdelE1hCMVminFL-nefBGHpA(s) was digested with restriction 20 enzymes Pac1 and Bst1107 I (or its isoschizomer, BstZ107 I) and then co-transformed into E. coli strain BJ5183 with linearized (Cla1 digested) adenoviral backbone plasmid, MRKpAd(E1/E3+)Cla1. The resulting pre-plasmid originally named MRKpdelE1hCMVminFL-nefBGHpA(s) is now referred to as "pMRKAd5nef". The genetic structure of the resulting pMRKAd5nef was verified by PCR, restriction 25 enzyme and DNA sequence analysis. The vectors were transformed into competent E. coli XL-1 Blue for preparative production. The recovered plasmid was verified by restriction enzyme digestion and DNA sequence analysis, and by expression of the nef transgene in transient transfection cell culture. The complete nucleotide sequence of this pMRKAd5HIV-1nef adenoviral vector is shown in Figure 27A-AM. 30

Generation of research-grade recombinant adenovirus - The pre-adenovirus plasmid, pMRKAd5nef, was rescued as infectious virions in PER.C6® adherent monolayer cell culture. To rescue infectious virus, 12 µg of pMRKAdnef was digested with restriction enzyme Pac1 (New England Biolabs) and 3.3 µg was transfected per 6 cm dish of PER.C6® cells using the calcium phosphate coprecipitation technique (Cell Phect Transfection Kit, Amersham Pharmacia Biotech

Inc.). Pac1 digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into PER.C6[®] cells. Infected cells and media were harvested 6-10 days post-transfection, after complete viral cytopathic effect (CPE) was observed. Infected cells and media were stored at ≤ -60°C. This nef containing recombinant adenovirus is now referred to as "MRKAd5nef".

EXAMPLE 21

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Construction of Murine CMV Promoter Containing Shuttle Vectors for Inactivated Pol and Nef/G2A,LLAA

The murine CMV (mCMV) was amplified from the plasmid pMH4 (supplied by Frank Graham, McMaster University) using the primer set: mCMV (Not I) Forward: 5'-ATA AGA ATG CGG CCG CCA TAT ACT GAG TCA TTA GG-3' (SEQ ID NO: 20); mCMV (Bgl II)Reverse: 5'-AAG GAA GAT CTA CCG ACG CTG GTC GCG CCT C-3' (SEQ ID NO:21). The underlined nucleotides represent the Not I and the Bgl II sites respectively for each primer. This PCR amplicon was used for the construction of the mCMV shuttle vector containing the transgene in the E1 parallel orientation. The hCMV promoter was removed from the original shuttle vector (containing the hCMV-gag-bGHpA transgene in the E1 parallel orientation) by digestion with Not I and Bgl II. The mCMV promoter (Not I/Bgl II digested PCR product) was inserted into the shuttle vector in a directional manner. The shuttle vector was then digested with Bgl II and the gag reporter gene (Bgl II fragment) was re-inserted back into the shuttle vector. Several clones were screened for correct orientation of the reporter gene. For the construction of the mCMV-gag in the E1 antiparallel orientation, the mCMV promoter was amplified from the plasmid pMH4 using the following primer set: mCMV (Asc I) Forward: 5'- ATA AGA ATG GCG CGC CAT ATA CTG AGT CAT TAG G (SEQ ID NO:22); mCMV (Bgl II) Reverse: 5' AAG GAA GAT CTA CCG ACG CTG GTC GCG CCT C (SEQ ID NO:23). The underlined nucleotides represent the Asc I and Bgl II sites, respectively for each primer. The shuttle vector containing the hCMV-gag transgene in the E1 antiparallel orientation was digested with Asc1 and Bgl11 to remove the hCMV-gag portion of the transgene. The mCMV promoter (Asc1/Bgl11 digested PCR product) was inserted into the shuttle vector in a directional manner. The vector was then digested with Bgl11 and the gag reporter gene (Bgl11 fragment) was re-inserted. Several clones were screened for correct orientation of the reporter gene. For each of the full length IA pol and full length nef/G2A,LLAA genes, cloning was performed using the unique

 $Bgl ext{ II}$ site within the mCMV-bGHpA shuttle vector. The pol and nef genes were excised from their respective pV1Jns plasmids by $Bgl ext{ II}$ digestion.

EXAMPLE 22

Construction of mCMV Full Length Inactivated Pol and Full Length nef/G2A.LLAA Adenovectors

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Each of these transgenes of Example 21 were inserted into the modified shuttle vector in both the E1 parallel and E1 anti-parallel orientations. Pac1 and BstZ110I digestion of each shuttle vector was performed and each specific transgene fragment containing the flanking Ad5 sequences was isolated and co-transformed with Cla I digested MRKpAd5(E3+) or MRKpAd5(E3-) adenovector plasmids via bacterial homologous recombination in BJ5183 E. coli cells. Recombinant preplasmid adenovectors containing the various transgenes in both the E3- and E3+ versions (and in the E1 parallel and E1 antiparallel orientations) were subsequently prepared in large scale following transformation into XL-1 Blue E. coli cells and analyzed by restriction analysis and sequencing.

EXAMPLE 23

Construction of hCMV-tpa-nef (LLAA) Adenovector

The tpa-nef gene was amplified out from GMP grade pV1Jns-tpanef (LLAA) vector using the primer sets: Tpanef (BamHI) F 5'-ATT GGA TCC ATG GAT GCA ATG AAG AGA GGG (SEQ ID 24); Tpanef (BamHI) R 5'-ATA GGA TCC TTA GCA GTC CTT GTA GTA CTC G (SEQ ID NO:25). The resulting PCR product was digested with BanHI, gel purified and cloned into the Bgl II site of MRKAd5CMV-bGHpA shuttle vector (Bgl II digested and calf intestinal phosphatase treated). Clones containing the tpanef (LLAA) gene (see SEQ ID NO:15 for complet coding region) in the correct orientation with respect to the hCMV promoter were selected following Sca I digestion. The resulting MRKAd5tpanef shuttle vector was digested with Pac I and Bst Z1101 and cloned into the E3+ MRKAd5 adenovector via bacterial homologous recombination techniques.

EXAMPLE 24

Immunogenicity of MRKAd5pol and MRKAd5nef Vaccine

Materials and Methods - Rodent Immunization - Groups of N=10 BALB/c

mice were immunized i.m. with the following vectors: (1) MRKAd5hCMV-IApol

(E3+) at either 10^7 vp and 10^9 vp; and (2) MRKAd5hCMV-IApol (E3-) at either

10^7 vp and 10^9 vp. At 7 weeks post dose, 5 of the 10 mice per cohort were boosted with the same vector and dose they initially received. At 3 weeks post the second does, sera and spleens were collected from all the animals for RT ELISA and IFNg ELIspot analyses, respectively. For all rodent immunizations, the Ad5 vectors were diluted in 5 mM Tris, 5% sucrose, 75 mM NaCl, 1 mM MgCl2, 0.005% polysorbate 80, pH 8.0. The total dose was injected to both quadricep muscles in 50 µL aliquots using a 0.3-mL insulin syringe with 28-1/2G needles (Becton-Dickinson, Franklin Lakes, NJ).

Groups of N=10 C57/BL6 mice were immunized i.m. with the following vectors: (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+) at either 10^7 vp and 10^9 vp; (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10^7 vp and 10^9 vp; and (3) MRKAd5mCMV-tpanef(LLAA) (E3+) at either 10^7 vp and 10^9 vp. At 7 weeks post dose, 5 of the 10 mice per cohort were boosted with the same vector and dose they initially received. At 3 weeks post the second does, sera and spleens were collected from all the animals for RT ELISA and IFNg ELIspot analyses, respectively.

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Non-human Primate immunization - Cohorts of 3 rhesus macaques (2-3 kg) were vaccinated with the following Ad vectors: (1) MRKAd5hCMV-IApol (E3+) at either 10^9 vp and 10^11 vp dose; and (2) MRKAd5hCMV-IApol (E3-) at either 10^9 vp and 10^11 vp; (3) MRKAd5hCMV-nef(G2A,LLAA) (E3+) at either 10^9 vp and 10^11 vp; and (4) MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10^9 vp and 10^11 vp. The vaccine was administered to chemically restrained monkeys (10 mg/kg ketamine) by needle injection of two 0.5 mL aliquots of the Ad vectors (in 5 mM Tris, 5% sucrose, 75 mM NaCl, 1 mM MgCl₂, 0.005% polysorbate 80, pH 8.0) into both deltoid muscles. The animals were immunized twice at a 4 week interval (T=0, 4 weeks).

Murine anti-RT and anti-nef ELISA - Anti-RT titers were obtained following standard secondary antibody-based ELISA. Maxisorp plates (NUNC, Rochester; NY) were coated by overnight incubation with 100 μL of 1 μg/mL HIV-1 RT protein (Advanced Biotechnologies, Columbia, MD) in PBS. For anti-nef ELISA, 100 uL of 1 ug/mL HIV-1 nef (Advanced Biotechnologies, Columbia, MD) was used to coat the plates. The plates were washed with PBS/0.05% Tween 20 using Titertek MAP instrument (Hunstville, AL) and incubated for 2 h with 200 μL/well of blocking solution (PBS/0.05% tween/1% BSA). An initial serum dilution of 100-fold was performed followed by 4-fold serial dilution. 100-μL aliquots of serially diluted samples were added per well and incubated for 2 h at room temperature. The plates

were washed and 100 μ L of 1/1000-diluted HRP-rabbit anti-mouse IgG (ZYMED, San Francisco, CA) were added with 1 h incubation. The plates were washed thoroughly and soaked with 100 μ L 1,2-phenylenediamine dihydrochloride/hydrogen peroxide (DAKO, Norway) solution for 15 min. The reaction was quenched by adding 100 μ L of 0.5M H₂SO4 per well. OD₄₉₂ readings were recorded using Titertek Multiskan MCC/340 with S20 stacker. Endpoint titers were defined as the highest serum dilution that resulted in an absorbance value of greater than or equal to 0.1 OD₄₉₂ (2.5 times the background value).

Non-human primate and murine ELIspot assays - The enzyme-linked immuno-spot (ELISpot) assay was utilized to enumerate antigen-specific INFγ-secreting cells from mouse spleens (Miyahira, et al.1995, J. Immunol. Methods 181:45-54) or macaque PBMCs. Mouse spleens were pooled from 5 mice/cohort and single cell suspensions were prepared at 5x10⁶/mL in complete RPMI media (RPMI1640, 10% FBS, 2mM L-glutamine, 100U/mL Penicillin, 100 u/mL streptomycin, 10 mM Hepes, 50 uM β-ME). Rhesus PBMCs were prepared from 8-15 mL of heparinized blood following standard Ficoll gradient separation (Coligan, et al, 1998, Current Protocols in Immunology. John Wiley & Sons, Inc.). Multiscreen opaque plates (Millipore, France) were coated with 100 μL/well of either 5 μg/mL purified rat anti-mouse IFN-γ IgG1, clone R4-6A2 (Pharmingen, San Diego, CA), or 15 ug/mL mouse anti-human IFN-γ IgG2a (Cat. No. 1598-00, R&D Systems, Minneapolis, MN) in PBS at 4°C overnight for murine or monkey assays, respectively. The plates were washed with PBS/penicillin/streptomycin and blocked with 200 μL/well of complete RPMI media for 37 °C for at least 2 h.

To each well, 50 μL of cell samples (4-5x10⁵ cells per well) and 50 μL of the antigen solution were added. To the control well, 50 μL of the media containing DMSO were added; for specific responses, either selected peptides or peptide pools (4 ug/mL per peptide final concentration) were added. For BALB/c mice immunized with the pol constructs, stimulation was conducted using a pool of CD4⁺-epitope containing 20-mer peptides (aa21-40, aa411-430, aa641-660, aa731-750, aa771-790) or a pool of CD8⁺-epitope containing peptides (aa201-220, aa311-330, aa781-800). For C57/BL6 mice immunized with the nef construct, either aa51-70 (CD8⁺ T cell epitope) or aa81-100 (CD4⁺) peptide derived from the nef sequence was added for specific stimulation. In monkeys, the responses against pol were evaluated using two pools (L and R) of 20-aa peptides that encompass the entire pol sequence and overlap by 10 amino acids. In monkeys vaccinated with the nef constructs, a single pool containing 20-mer peptides covering the entire HIV-1 nef sequence and overlapping

by 10 aa was used. Each sample/antigen mixture was performed in triplicate wells for murine samples or in duplicate wells for rhesus PBMCs. Plates were incubated at 37°C, 5% CO₂, 90% humidity for 20-24 h. The plates were washed with PBS/0.05% Tween 20 and incubated with 100 μL/well of either 1.25 μg/mL biotin-conjugated rat anti-mouse IFN-γ mAb, clone XMG1.2 (Pharmingen) or of 0.1 μg/mL biotinylated anti-human IFN-gamma goat polyclonal antibody (R&D Systems) at 4°C overnight. The plates were washed and incubated with 100 μL/well 1/2500 dilution of strepavidin-alkaline phosphatase conjugate (Pharmingen) in PBS/0.005% Tween/5% FBS for 30 min at 37 °C. Spots were developed by incubating with 100 μL/well 1-step NBT/BCIP (Pierce Chemicals) for 6-10 min. The plates were washed with water and allowed to air dry. The number of spots in each well was determined using a dissecting microscope and the data normalized to 10⁶ cell input.

Non-human Primate anti-RT ELISA - The pol-specific antibodies in the monkeys were measured in a competitive RT EIA assay, wherein sample activity is determined by the ability to block RT antigen from binding to coating antibody on the plate well. Briefly, Maxisorp plates were coated with saturating amounts of pol positive human serum (#97111234). 250 uL of each sample is incubated with 15 uL of 266 ng/mL RT recombinant protein (in RCM 563, 1% BSA, 0.1% tween, 0.1% NaN₃) and 20 uL of lysis buffer (Coulter p24 antigen assay kit) for 15 min at room temperature. Similar mixtures are prepared using serially diluted samples of a standard and a negative control which defines maximum RT binding. 200 uL/well of each sample and standard were added to the washed plate and the plate incubated 16-24 h at room temperature. Bound RT is quantified following the procedures described in Coulter p24 assay kit and reported in milliMerck units per mL arbitrarily defined by the chosen standard.

Results - Rodent Studies - BALB/c mice (n=5 mice/cohort) were immunized once or twice with varying doses of MRKAd5hCMV-IApol(E3+) and MRKAd5hCMV-IApol(E3-). At 3 weeks after the second dose, Anti-pol IgG levels were determined by an ELISA assay using RT as a surrogate antigen. Cellular response were quantified via IFNy ELISpot assay against pools of pol-epitope containing peptides. The results of these assays are summarized in Table 10. The results indicate that the mouse vaccinees exhibited detectable anti-RT IgGs with an adenovector dose as low as 10^7 vp. The humoral responses are highly dose-dependent and are boostable with a second immunization. One or two doses of either pol vectors elicit high frequencies of antigen-specific CD4⁺ and CD8⁺ T cells; the responses are weakly dose-dependent but are boostable with a second immunization.

Table 10. Immunogenicity of MRKAd5pol Vectors in BALB/c mice.

				An	II-RT IgG Tite	rs°	S	FC/10^6 cell	s <u> </u>
Group	Vaccine	Dose	No. of Doses	GMT	+SE	-SE	Medlum	CD4+ peptide pool	CD8+ peptide pool
1	MRKAdShCMVFLpal (E3+)	10^7 vp	2 1	310419 919	301785 372	153020 265	1(1) 1(1)	75(4) 72(9)	2313(67) 533(41)
2	MRKAdShCMVFLpol (E3+)	10^9 vp	2	1638400 ^b 713155	0 528520	0 303555	2(2) 1(1)	114(9) 48(7)	2063(182) 733(89)
3	MRKAd5hCMVFLpol (E3-)	10^7 vp	2	310419 6400	38821B 14013	172097 4393	0(0) 10(8)	223(7) 141(21)	2607(27) 409(28)
4	MRKAd5hCMVFLpol (E3-)	10^9 vp	2	1838400 ^b 1241675 ^b	0 396725	0 · 300681	1(1) 0(0)	160(13) 39(13)	2385(11) 833(83)
	Naïve	none	none	57	9	7	9(2)	11(4)	10(1)

^{*}GMT, geometric mean titer of the cohort of 5 mice; SE, standard error of the gemetric mean

5 C57/BL6 mice were immunized once or twice with varying doses of MRKAd5hCMV-nef(G2A,LLAA) (E3+), MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10^7 vp and(3) MRKAd5mCMV-tpanef(LLAA) (E3+) at either 10^7 vp and 10^9 vp. The immune response were analyzed using similar protocols and the results are listed in Table 11. While anti-nef IgG responses could not be detected in this model system with any of the constructs, there are strong indications of a cellular immunity generated against nef using the ELIspot assay.

Table 11. Immunogenicity of MRKAd5nef Vectors in C57/BL6 mice.

				Ar	ti-nef IgG Tite	ers"	S	FC/10^6 cell	5
Group	Vaccine	Dose	No. of Doses	GMT	+SE	-SE	Medium	2251-70 CD8+	8881-100 CD4+
	MRKAd5hCMVFLnel (E3+)	10^7 vp	2	174	70	50	1(1)	23(1)	1(1)
•	tarao estretir e la (-e.)		1	132	42	32	0(0)	0(0)	0(0)
	MRKAd5hCMVFLnel (E3+)	10^9 Vp	2	174	70	50	0(0)	61(7)	4(2)
•	(A		1	132	42	32	1(1)	62(7)	3(1)
3	MRKAd5mCMVFLnet (E3+)	10^7 VD	2	132	42	32	3(1)	15(5)	5(2)
	, , , , , , , , , , , , , , , , , , ,		1 -	115	46	33	3(2)	3(2)	4(2)
· ·	MRKAd5mCMVFLnef (E3+)	10^9 VD	2	132	42	32	4(2)	83(13)	5(1)
•			1	132	42	32	2(1)	28(2)	4(0)
5	MRKAd5mCMVtpanel(E3+)	10^7 VD	2	132	42	32 .	3(2)	14(2)	5(1)
_	, , , , , , , , , , , , , , , , , , , ,] '	1	100	0	0	3(1)	13(4)	10(3)
6	MRKAd5mCMVtpanet(E3+)	10^9 Vp	2	230	170	98	3(2)	145(29)	4(0)
	# * (,		1	115	46	33	7(1)	151(14)	10(0)
7	Naïve	none	none	152	78	52 ·	21(2)	- 18(6)	28(3)

[&]quot;GMT, geometric mean titler of the cohort of 5 mice; SE, standard error of the gemetric mean

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Monkey Studies - Cohorts of 3 rhesus macaques were immunized with 2 doses of MRKAd5hCMV-IApol(E3+) and MRKAd5hCMV-IApol(E3-). The number of antigen-specific T cells (per million PBMCs) were enumerated using one of two

Near or at the upper limit of the serial dilution; hence, could be greater than this value

[°]No. of Spot-forming Cells per millon splechoytes; mean values of triplicates are reported along with standard errors in parenthesis.

No. of spol-forming cells per million spiecnoytes; mean values of triplicates are reported along with standard errors in parenthesia.

peptide pools (L and R) that cover the entire pol sequence; the results are listed in Table 12. Moderate-to-strong T cell responses were detected in the vaccinees using either constructs even at a low dose of 10^9 vp. Longitudinal analyses of the anti-RT antibody titers in the animals suggest that the pol transgene product is expressed efficiently to elicit a humoral response (Table 13). It would appear that generally higher immune responses were observed in animals that received the E3- construct compared to the E3+ virus.

Table 12. Pol-specific T Cell Responses in MRKAd5pol Immunized Rhesus

10 Macaques.

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Vaccine (T=0,4 wks)	Monk #		Prebleed			T=4			T=7			T=16	
		Mock	Pol L	PolR	Mock	Pol L	Pol R	Mock	Pol L	Pol R	Mock	Pol L	Pol R
MRKAdShCMV-I Apol(E3+)	990100	1	0	0	1	38	31	0	52	146	0	49	715
10^11 VD	99C215	1 1	2	2	10	98	249	1	109	305	22	88	250
	99D201	5	5	4	6	149	85	0	40	35	٥	35	18
MRKAd6hCMV-IApol(E3+)	99D212	0	2	0	4	331	114	0	58	14	0	6	6
10'9 vp	99D18D	0	4	2	0	19	192	4	38	158	5	38	108
	99C201	8	5	21	В	62	62	0	18	32	١,	14	65
MRKACENCMV-I Apod (E3-)	990239	5	2	2	20	82	172	1	68	114	9	21	- 40
10/11 vp	99C186	4	12	6	5	120	421	2	271	489	16	875	530
·	990084	1	8	9	8	84	464	٥	14	238	1	24	264
MRKAd5hCMV-IApol(E3-)	007C	10	10	8	12	724	745	4	322	376	4	188	176
10/9 vp	CDIG	2	0	1	5	474	468	0	232	212	0	101	121
	CD 11	6	6	12	10	98	110	5	60	80	8	25	34
Nave	083Q	nd	nd	nd	nd	nd	nd	4	2	2_	2	1	2

nd, not determined Reported are SFC per million PBMCs; mean of duplicate wells.

Table 13. Anti-RT Ig Levels in MRKAd5pol Immunized macaques.

RT ANTIBODY ASSAYTITERS IN mMU/ Voccine/MonkeyTog	T=4	T=7	T=12	T=16
MRKAd5hCMV-IApol(E3+), 10^11 vp				
99C100	61	1999	5928	4768
99C215	81	1541	2356	2767
99D201	53	336	539	387
MRKAd5hCMV-IApol(E3+), 10^9 vp			·	
99D212	10	40	49	68
99D180	<10	36	79	93
99C201	<10	37	71	76
MRKAd5hCMV-IApol(E3-), 10^11 vp	 			
99D239	44	460	1234	1015
99C186	21	· 233 ·	480	345
990084	235	2637	2858	1626
MRKAd5hCMV-lApol(E3-), 10^9 vp	 			
OC7C	32	175	306	235
©16	20	140	273	419
CD11	15	112_	149	237

When rhesus macaques were immunized i.m. with two doses of MRKAd5nef constructs, vigorous T cell responses ranging from 100 to as high as 1100 per million were observed in 8 of 12 vaccinees (Table 14). The efficacies of the mCMV- and hCMV- driven nef constructs are comparable on the basis of the data generated thus far.

Table 14. Nef-specific T cell Responses in MRKAd5nef Immunized Rhesus

Macaques.

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Vaccine (T=0,4 wks)	Monk #	Pi	ne _	T	-4	T	:7	T	16
		Mock	Nef	Mock	Nef	Mock	Net	Mock	Nef
MRKAd5hCMV-nef(G2A,LLAA) (E3+)	CD2D	0	4	31	440	4	368	1	251
10^11 vp	CC7B	0	0	2	521	0	178	1	152
•	CC61	2	9	31	112	0	108	-11	100
MRKAd5hCMV-nef(G2A,LLAA) (E3+)	CC2K	9	9	6	52	0	35	0	15
10^9 vp	CD15	5	4	30	998	2	586	0	434
	CD16	6	1	6	1146	0	369	1	21:
MRKAd5mCMV-nef(G2A,LLAA) (E3+)	99D191	1	5	4	614	0	298	2	41
10^11 vp	99D144	4	6	5	434	0	1100	2	93:
•	99C193	1	2	1	58	1	22	0	64
MRKAd5mCMV-nef(G2A,LLAA) (E3+)	99D224	1	11	14	231	1	125	0	70
10/9 vp	99D250	8	. 9	4	108	0	54	0	5
	990120	1	6	20	299	0	92	0	71
Naive	083Q	nd	nd	1B	22	4	5	2	1

EXAMPLE 25

Comparison of Clade B vs. Clade C T Cell Responses in HIV-Infected Subjects

PBMC samples collected from two dozens of patients infected with HIV-1 in

US were tested in ELISPOT assays with peptide pools of 20-mer peptides overlapping
by 10 amino acids. Four different peptide pools were tested for cross-clade

recognition, and they were either derived from a clade B-based isolate (gag H-b; nefb) or a clade C-based isolate (gag H-c, nef-c). Data in Table 15 shows that T cells

from these patients presumably infected with clade B HIV-1 could recognize clade C
gag and nef antigens in ELISPOT assay. Correlation analysis further demonstrated
that these T cell responses against clade C gag peptide pool were about 60% of the
clade B counterpart (Figure 24), while the T cell responses against clade C nef were
about 85% of the clade B counterpart (Figure 25). These results suggest that cellular
immune responses generated in patients infected with clade B HIV-1 can recognize
gag and nef antigens derived from clade C HIV-1. These data show that a HIV
vaccine, such as a DNA or MRKAd5-based adenoviral vaccine expressing a clade B

gag and/or nef antigen will potentially have the ability to provide a prophylactic and/or therapetic advantage on a global scale.

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Table 15
Responses Shown as the Number of gIFN-Secreting T Cells per Million PBMCs

subject	bleed date	gag epitope #	mock	gag H-b	gagH-c	nef-b	nef-c
		from mapping)					
#100	19-Jul-99	12	10	3950	1385	1295	1300
#101	25-Jul-99	3	15	3885	1280	na	1020
#102	25-Jul-99	4	15	1740	850	1255	1785
#104	7-Jun-99	2	. 5	1355	1185	na	1060
#107	11-Oct-99	2	25	3305	2795	670	870
#405	11-Jul-99	2	15	4575	3180	1700	1500
#501	19-Jul-99	2	15	1100	570	3365	3460
#505	18-Jul-99	5	10	2145	1725	1235	na
#506	28-Feb-99	2	25	150	45	400	610
#701	28-Mar-99	5	30	7620	4775	3320	2780
#709	17-May-99	3	15	2785	1945	1090	1630
#710	24-May-99	4	5	1055	1080	2210	2140
•	1						

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EXAMPLE 26

Characterization and Production of MRKAd5pol and MRKAd5nef Vectors in Roller Bottles

Expansion of nef and pol Adenovectors - Nef and pol CsCl purified MRKAd5 seeds were used to infect roller bottles to produce P4 virus to be used as a seed for further experiments. P4 MRKAd5 pol and nef vectors were used to infect roller bottles at an MOI 280 vp/cell, except for hCMV-tpa-nef [E3+] which was infected at an MOI of 125 due to low titers of seed obtained at P4.

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Table 16 Viral particle concentrations for P5 nef and pol adenovectors

Adenovector	AEX Titer (10 ¹⁰ vp/ml culture)	AEX Titer (10 ⁴ vp/cell)	Amplification Ratio
hCMV-FL-nef [E3+]	1.1	0.9	30
mCMV-FL-nef [E3+]	2.2	2.1	75
hCMV-tpa-nef [E3+]	0.07	0.1	5
mCMV-tpa-nef [E3+]	1.3	0.9	35
hCMV-FL-pol [E3+]	2.7	2.1	75
hCMV-FL-pol [E3-]	1.9	1.3	45

Roller Bottle Passaging - Passaging of the pol and nef constructs continued through passage seven. Cell-associated (freeze/thaw lysis) and whole broth (tritonlysis) titers obtained in all passages were very consistent. In general, MRKAd5pol is ca. 70% as productive as MRKAd5gag while MRKAd5nef is ca. 25% as productive as MRKAd5gag. Samples of P7 virus for both constructs were analyzed by V&CB by restriction digest analysis and did not show any rearrangements.

Table 17. Passage Six Viral Productivity for MRKAd5pol and MRKAd5nef

1 autc	-·· -		Xviable (10° cells/ml),		AEX Titer	Tites	Amplification	Triton Lysis Titer
		Viahil Infection	ity (%) Harvest	Number	(Cell Associated) 10 ¹⁰ vp/ml culture	104 vp/cell	Ratio	10 ¹⁰ vp/ml culture
hCMV-FL-nsf [B3+]	pool	1.22, 85%		62	0.8	0.7	25	1.6
	 1		0.99, 62%	1				
	2		1.10,72%	1	}			
bCMV-FL-pol [E3+]	pool	1.42, 89%		62	4.5	3.2	115	7.0
	. 1		1.22, 70%					
	2		1.42, 74%					

15 Table 18. Passage Seven Viral Productivity for MRKAd5pol and MRKAd5nef

		Xviable (10 Viabili Infection) ⁶ cells/ml),	Cell Passage Number	AEX Titer (Cell Associated) 10 ¹⁰ vp/ml culture	Titer 10 ⁴ vp/cell	Amplification Ratio	Triton Lysis Titer 10 ^m vp/ml culture
hCMV-FL-nef (E3+)	Pool	1.33, 90%		66	1.0	0.8	29	2.1
	- 1		0.96, 70%					
	2		1.18, 73%	.	1			
bCMV-FL-pol [E3+]	Pool	0.90*, 90%		56	4.2	4.7	168	6.5
	1		1.18, 88%					
	2	'	1.04, 80%	<u> </u>			<u> </u>	

MRKAd5nef and MRKAd5pol Viral Production Kinetics - A timecourse experiment was carried out in roller bottles to determine if the viral production kinetics of the MRKAd5pol and MRKAd5nef vectors were similar to those of MRKAd5gag. PER.C6® cells in roller bottle cultures were infected at an MOI of 280 vp/cells with P5 MRKAd5pol, P5 MRKAd5nef and P7 MRKAd5gag; for each adenovector, two infected bottles were sampled at 24, 36, 48, and 60 hours post infection. In addition, two bottles were left unsampled until 48 hpi when they were harvested under the Phase I process conditions. The anion-exchange HPLC viral particle concentrations of the freeze-thaw recovered cell associated virus at the 24, 36,

48, and 60 hpi timepoints are shown in Figure 29A-B. The QPA titers show a similar trend (data not shown).

Comparison of hCMV- and mCMV-FL-nef - As the titers obtained with the MRKAd5nef construct (hCMV-FL-nef) were lower than those obtained with MRKAd5gag or MRKAd5pol, a viral productivity comparison experiment was performed with mCMV-FL-nef. For each of the two adenovectors (hCMV- and mCMV-FL-nef), two roller bottles were infected at an MOI of 280 vp/cell with passage five clarified lysate. The macroscopic and microscopic observations of the four roller bottles were identical at the time of harvest. Analysis of the clarified lysate produced indicated a higher viral particle concentration in the bottles infected with mCMV-FL-nef, as shown in Table 19. It is stipulated that the higher productivity with mCMV promoter driven nef vector is due to lower nef expression levels in PER.C6® cells- experiments are underway at V&CB to measure nef expression levels.

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Table 19. Passage Six Viral Productivity Comparison of hCMV- and mCMV-FL-nef

	(Xv (10 ⁶ cells/m	l), Viability (%)	Cell Passage	AEX Titer	Titer	Amplification	Triton Lysis Titer
		Infection	Harvest	Number	10 ¹⁰ vp/ml culture	10 ⁴ vp/cell	Ratio	10 ^{to} vp/ml culture
hCMV-FL-nef	Pool	1.11, 91%		60	1.5	1.4	50	2.8
(MRKAdSnef)	. 1		1.23,75%					
	2	i	1.34,74%					
mCMV-FL-nef	Pool	1.11, 91%		60	2.3	2.1	75	4.6
	1		1.49, 84%					
	2		1.18,77%					

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EXAMPLE 27

Characterization and Large Scale Production of MRKAd5nef Virus in Bioreactors

Materials and Methods - The experiment of the present example was run twice under the following conditions: 36.5°C, DO 30%, pH 7.30, 150rpm agitation rate,

25 no sparging, Life Technologies (Gibco, Invitrogen) 293 SFM II (with 6mM L-glutamine), 0.5M NaOH as base for pH control. During the first run (B20010115), two 10L stirred vessel bioreactors were inoculated with PER.C6® cells at a concentration of 0.2x106 cells/ml. Cells were grown until they reached a cell concentration of approximately 1x106 cells/ml. The cells were infected with uncloned

30 MRKAd5nef (G2A,LLAA) at a MOI of 280 virus particles (vp)/cell. For the second batch (B20010202), the same procedure as the first run was used, except the cells

were infected with cloned MRAd5nef. During both runs, the bioreactors were harvested 48 hours post-infection. Samples were taken and virus concentrations were determined from whole broth (with triton lysis), supernatant, and cell pellets (3 X freeze/thaw) with the AEX and QPA assays. Metabolites were measured with BioProfile 250 throughout the process.

Table 20: Experimental Conditions

Temperature	- 36.5 ℃
DO	30%
PH	7.30
Agitation	150 rpm
Sparging	None

Table 21: Virus source used for experiments.

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Run	Batch ID	Cloned/Uncloned MRKAd5nef	MOI (vp/cells)
#1	B20010115-1	Uncloned	280
	B20010115-2	Uncloned	280
#2	B20010202-1	Cloned	280
	B20010202-2	Cloned	280

Results - Table 22 and 23 show an the ability to scale up production of MRKAd5nef by growth in a bioreactor.

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Table 22: Virus Concentration as measured by the AEX assay

Run	Batch ID	Cloned/Uncloned	Virus Concentration @ 48hpi (1x10 ¹³ vp/L)							
		MRKAd5nef	Supernatant	Clarified Lysate	Total	Triton Lysate				
#1	B20010115-1	Uncloned	0.72	3.26	3.98	5.76				
	B20010115-2	Uncloned	0.38	1.67	2.05	2.46				
#2	B20010202-1	Cloned	0.80	6.00	6.80	8.88				
"-	B20010202-2	Cloned	0.50	6.00	6.50	8.47				

Table 23: Virus Titers as measured by the QPA assay

Run	Batch ID	Cloned/Uncloned	Virus Concentration @ 48hpi (1x10 ¹¹ IU/L)							
	·	MRKAd5nef	Whole Broth	Supernatant	Clarified Lysate	Total	Triton Lysate			
#1	B20010115-1	Uncloned	0.13	1.12	1.76	2.88	11.28			
"-	B20010115-2	Uncloned	0.14	0.73	1.54	2.27	5.86			
#2	B20010202-1	Cloned	0.14	0.97	1.62	2.69	11.89			
"-	B20010202-2	Cloned	0.14	1.17	1.70	2.97	12.47			

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The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art

from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

EXAMPLE 28

MRKAd5HIV-1gag Boosting of DNA-Primed Animals

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Groups of 3-5 rhesus macaques were immunized with (a) 5 mgs of V1Jns-Flgag (pVIJnsCMV(no intron)-FL-gag-bGHpA), (b) 5 mgs of V1Jns-Flgag formulated with 45 mgs of a non-ionic block copolymer CRL1005, or (c) 5 mgs of V1Jns-Flgag formulated with 7.5 mgs of CRL1005 and 0.6 mM benzalkonium chloride at weeks 0, 4, and 8. All animals received a single dose of 10e7 viral particles (vp) of the MRKAd5HIV-1gag at week 26. Note: 10e7 is too low to prime or boost effectively when used as a single modality (dose is selected to mimic preexposure to adenovirus); see Figure 32.

Blood samples were collected from all animals at several time points and peripheral blood mononuclear cells (PBMCs) were prepared using standard Ficoll method. The PBMCs were counted and analyzed for gamma-interferon secretion using the ELISpot assay (Table 24). For each monkey, the PBMCs were incubated overnight either in the absence (medium) or presence of a pool (called "gag H") of 50 20-aa long peptides that encompass the entire HIV-1 gag sequence.

The results indicate that MRKAd5HIV-1gag was very effective in boosting the T cell immune responses in these monkeys. At week 28 or 2 weeks after the viral boost, the number of gag-specific T cells per million PBMCs increased 2-48 fold compared to the levels observed at week 24 or 2 weeks prior to the boost.

The PBMCs were also analyzed by intracellular gamma-interferon staining prior to (at week 10) and after the MRKAd5gag boost (at week 30). The results for select animals are shown on Figure 31. The results indicate that (a) immunization with DNA/adjuvant formulation elicited T cell responses which can either be balanced, CD4⁺-biased or CD8⁺-biased, and (b) boosting with the MRKAd5gag construct produced in all cases a strongly CD8⁺-biased response. These results suggest that boosting with MRKAd5HIV-1gag construct is able to improve the levels of antigen-specific CD8⁺ T cells.

Monte Tag	Medium	¥	0	AW3G 5		SCIC 0	OCIK 4	6	×	AK88 9 12		AW20 10 4	_	6 828	OSSW 4	CB70	980201 3 0
12	Mediu	-	-	98		8	-	-	•	28		1 59	3 121	9	8	136	0 0
746	Mediu	19	_	3 61		111	0 284	7	0 288	1 119	_	5 284	135	3 119		316	0 1
Toto	Medium gag H	122 1 1	0	97		5 270	0 781	4 164	630	0 439		19 425	_	0 278	0 138	606	0 0
1017	Medhan gap H	8 115	_	2		4 280	6 452		. 19 374	_		9	- S	8	- 0	5 629	0 1
T=24	Medium gag H	9 05	0 32	_		8 222	0	98	B 251	0 316		8 208	1 105	1 209	- 62	1 769	2
1=28	Medium gap H	19 956	_	_	4	38 886	0 191	=======================================	8 154	4 1229		18 585		-	. 64	0 22	9
1=30	+ Medium	0	-	•	1	49	_	_	8	_		8	_	-	-	•	٥
	E E	318	755	382		1345	8	241	25.	2 8		404	978	828	88	1831	۰

EXAMPLE 29

Construction of gagpol fusion for MRKAd5gagpol fusion constructs

The open reading frames for the codon-optimized HIV-1 gag gene was fused directly to the open reading frame of the IA pol gene (consisting of RT, RNAseH and integrase domains) by stepwise PCR. Because the gene (SEQ ID NO: 38) does not include the protease gene and the frameshift sequence, it encodes a single polypeptide of the combined size of p55, RT, RNAse H and integrase (1350 amino acids; SEQ ID NO: 39).

The fragment that extends from the BstEII site within the gag gene to the last non-stop codon was ligated via PCR to a fragment that extends from the start codon of the IApol to a unique BamHI site. This fragment was digested with BstEII and BamHI. Construction of gag-IApol fusion was achieved via three-fragment ligation involving the PstI-BstEII gag digestion fragment, the BstEII/BamHI digested PCR product and long PstI/BamHI V1R-FLpol backbone fragment.

The MRKAd5-gagpol adenovirus vector was constructed using the BglII fragment of the V1R-gagpol containing the entire ORF of gag-IApol fusion gene.

EXAMPLE 30

Immunogenicity Studies in Non-Human Primates

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Cohorts of three (3) macaques were immunized with 10e8 or 10e10 viral particles (vp) of one of the following MRKAd5 HIV-1 vaccines: (1) MRKAd5gag; (2) MRKAd5pol; (3) MRKAd5nef; (4) a mixture containing equal amounts of MRKAd5gag, MRKAd5pol, and MRKAd5nef, or (5) a mixture of equal amounts of MRKAd5gagpol and MRKAd5nef. The vaccines were administered at weeks 0 and 4.

The T cell responses against each of the HIV-1 antigens were assayed by IFN-gamma ELISpot assay using pools of 20-aa peptides that encompass the entire protein sequence of each antigen. The results (Table 25) are expressed as the number of spot-forming cells (sfc) per million peripheral blood mononuclear cells (PBMC) that respond to each of the peptide pools.

Results indicate the following observations: (1) each of the single gene constructs (MRKAd5gag, MRKAd5pol, or MRKAd5nef) is able to elicit high levels of antigen-specific T cells in monkeys; (2) the single-gene MRKAd5 constructs can be mixed as a multi-cocktail formulation capable of eliciting very broad T cell responses against gag, pol, and nef; (3) the MRKAd5 vector expressing the fusion

protein of gag plus IA pol is capable of inducing strong T cell responses to both gag and pol.

Table 25. Evaluation of Mixtures of MRKAd5 vectors expressing humanized

HIV-1 gag, pol, gagpol, nef in rhesus macaques

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Grp#	Vaccine	Monk #			T=6 wks		
	T=0, 4 wks		Mock	Gag H	Pol - 1	Pol - 2	Nef
1	MRKAd5 gag	CB9V	0	15	•	-	•
	10^10 vp	CD19	0 .	374			•
		109H	1	843	-	•	•
2	MRKAd5 gag	99D130	1	948	•	•	
	10^8 vp	W277	16	324	•	•	-
		143H	4	595	-	-	•
3	MRKAd5 pol	CC1X	4	•	46	256	•
-	10^10 vp	AW3W	3	-	463	550	-
		AV43	6	-	95	1333	-
4	MRKAd5 pol	AW38	1		19	30	-
1	10^8 vp	CC8K	0		50	995	-
		CC21	1	-	33	436	-
5	MRKAd5 nef	076Q	9	-	-	1	1204
_	10^10 vp	091Q	4	-	-	-	85
		083Q	0	-	-		176
6	MRKAd5 nef	00C029	1	-	-	-	114
	10^8 vp	98D022	6		-	-	170
		98D160	3	-	-	-	198
7	MRKAd5gag+MRKAd5pol+MRKAd5nef	99D251	3	206	15	193	120
	10^10 vp each	05H	3	135	21	9	638
- 1		00C016	3	26	4	51	23
- 8	MRKAd5gag+MRKAd5pol+MRKAd5nef	99D215	1	171	18	193	240
	10^8 vp each	81H	5	73	6	14	243
		12H	8	1140	115	811	719
9	MRKAd5gagpol +MRKAd5 nef	99D211	0	83	56	838	725
	10^10 vp each	22H	4	385	119	1194	1915
		61H	4	343	11	765	853
10	MRKAd5gagpol +MRKAd5 nef	34H	3	78	19	5	75
	10^8 vp each	48H	1	65	105	46	43
		70H	5	158	l 15	220	191

Indicated are numbers of spot-forming cells per million PBMCS against the peptide pools. Mock, no peptides; gag H, fifty 20-aa peptides encompassing p55 sequence; pol-1, 20-aa peptides representing N-terminal half of IA pol; pol-2, 20-aa peptides representing the carboxy-terminal half of IA pol; nef, 20-aa peptides encompassing the entire wild-type nef sequence. Responses to the antigens prior to the first immunization did not exceed 40 sfc/10%6 PBMC.

WHAT IS CLAIMED IS

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A recombinant adenoviral vaccine vector at least partially deleted in
 E1 and devoid of E1 activity, comprising:

- a) an adenovirus cis-acting packaging region corresponding to from about base pair 1 to between from about base pair 400 to about base pair 458 of a wildtype adenovirus genome; and
- b) a gene encoding an HIV protein or immunologically relevant modification thereof.
- A vector in accordance with claim 1 comprising a packaging region corresponding to from about base pair 1 to about base pair 450 of a wildtype adenovirus genome.
- 3. A vector in accordance with claim 1 further comprising nucleotides
 15 corresponding to between from about base pair 3511 to about 3524 to about base pair
 5798 of a wildtype adenovirus genome.
 - 4. A vector in accordance with claim 3 comprising base pairs corresponding to 1-450 and 3511-5798 of a wildtype adenovirus genome.
- 5. A vector in accordance with claim 4 which is deleted of base pairs451-3510.
 - 6. A vector in accordance with claim 1 which is at least partially deleted in E3.
 - 7. A vector in accordance with claim 6 wherein the E3 deleted region is from base pairs 28,133-30,818.

8. A vector in accordance with claim 1 wherein the gene encoding the HIV protein or modification thereof comprises codons optimized for expression in a human.

- 9. A vector in accordance with claim 1 wherein the vector comprises a gene expression cassette comprising:
 - a) a nucleic acid encoding a protein;
- b) a heterologous promoter operatively linked to the nucleic acid encoding the protein; and
 - (c) a transcription termination sequence.
- 10. A vector in accordance with claim 9 wherein the gene expression cassette is inserted into the E1 region.
 - 11. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette is in an E1 parallel orientation
- 12. An adenoviral vector in accordance with claim 9 wherein the geneexpression cassette is in an E1 antiparallel orientation.
 - 13. An adenoviral vector in accordance with claim 9 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.
 - 14. An adenoviral vector in accordance with claim 13 wherein the promoter is an immediate early human cytomegalovirus promoter.
- 20 15. An adenoviral vector in accordance with claim 9 wherein the promoter is a murine cytomegalovirus promoter.
 - 16. An adenoviral vector in accordance with claim 9 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

17. An adenoviral vector in accordance with claim 9 wherein the transcription termination sequence is a synthetic polyadenylation signal (SPA).

- 18. A cell comprising the adenoviral vector of claim 1.
- 19. Recombinant, replication-defective adenovirus particles harvested
 and purified subsequent to transfection of the adenoviral vector of claim 1 into a cell
 line which expresses adenovirus E1 protein at complementing levels.
 - 20. An HIV vaccine composition comprising purified adenovirus particles of claim 19.
- 21. An HIV vaccine composition of claim 20 which comprises aphysiologically acceptable carrier.
 - 22. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 1 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.
 - 23. A method according to claim 22 wherein the cell is a PER.C6® cell.

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- 24. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 21.
 - 25. A method according to claim 24 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

26. A method according to claim 25 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

- 27. A method according to claim 24 wherein the adenovirus vaccine is
 5 preceded by an adenovirus vaccine of a different serotype.
 - 28. A method according to claim 24 which comprises administering and readministering the adenovirus vaccine vector to the individual.
 - 29. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV gag or an immunologically relevant modification thereof.
- 30. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV gag protein or immunologically relevant modification thereof.
 - 31. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:
- a) an adenovirus cis-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and
- 20 b) a gene expression cassette comprising
 - i) SEQ ID NO: 29;
 - ii) a heterologous promoter operatively linked to i); and
 - iii) a transcription termination sequence.

32. An adenoviral vector in accordance with claim 31 wherein the gene expression cassette is in an E1 parallel orientation.

- 33 An adenoviral vector in accordance with claim 31 wherein the gene expression cassette is in an E1 antiparallel orientation.
- 34. An adenoviral vector in accordance with claim 31 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

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- 35. An adenoviral vector in accordance with claim 31 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.
- 36. An adenoviral vector in accordance with claim 31 which is at least partially deleted in E3.
 - 37. A cell comprising the adenoviral vector of claim 30.
 - 38. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 30 into a cell line which expresses adenovirus E1 protein at complementing levels.
 - 39. An HIV vaccine composition comprising purified adenovirus particles of claim 38.
 - 40. An HIV vaccine composition of claim 39 which comprises a physiologically acceptable carrier.
- 41. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 30 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

42. A method according to claim 41 wherein the cell is a PER.C6® cell.

43. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 21.

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- 44. A method according to claim 43 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.
- 45. A method according to claim 44 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.
 - 46. A method according to claim 43 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.
 - 47. A method according to claim 43 which comprises administering and readministering the adenovirus vaccine vector to the individual.
 - 48. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV pol or an immunologically relevant modification thereof.
- 49. An adenoviral vector in accordance with claim 9 wherein the gene
 20 expression cassette comprises an open reading frame encoding an HIV pol protein or immunologically relevant modification thereof.
 - 50. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

a) an adenovirus cis-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and

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- b) a gene expression cassette comprising
 - a nucleotide sequence selected the group consisting of SEQ ID NO: 1, SEQ ID NO: 5 and SEQ ID NO: 7;
 - ii) a heterologous promoter operatively linked to i); and
 - iii) a transcription termination sequence.
- 51. An adenoviral vector in accordance with claim 50 wherein the gene expression cassette is in an E1 parallel orientation.
- 52. An adenoviral vector in accordance with claim 50 wherein the gene expression cassette is in an E1 antiparallel orientation.
- 53. An adenoviral vector in accordance with claim 50 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.
- 54. An adenoviral vector in accordance with claim 50 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.
- 55. An adenoviral vector in accordance with claim 50 which is at least partially deleted in E3.
 - 56. A cell comprising the adenoviral vector of claim 49.

57. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 49 into a cell line which expresses adenovirus E1 protein at complementing levels.

- 58. An HIV vaccine composition comprising purified adenovirusparticles of claim 57.
 - 59. An HIV vaccine composition of claim 58 which comprises a physiologically acceptable carrier.
 - 60. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 49 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

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- 61. A method according to claim 60 wherein the cell is a PER.C6® cell.
- 62. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 59.
 - 63. A method according to claim 62 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.
 - 64. A method according to claim 63 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

65. A method according to claim 62 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

- 66. A method according to claim 62 which comprises administering and readministering the adenovirus vaccine vector to the individual.
- 67. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV nef or an immunologically relevant modification thereof.

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- 68. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV nef protein or immunologically relevant modification thereof.
- 10 69. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:
 - a) an adenovirus cis-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and
 - b) a gene expression cassette comprising
 - a nucleotide sequence selected the group consisting of SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13 and SEQ ID NO: 15;
 - ii) a heterologous promoter operatively linked to i); and
 - iii) a transcription termination sequence.
 - 70. An adenoviral vector in accordance with claim 69 wherein the gene expression cassette is in an E1 parallel orientation.

71. An adenoviral vector in accordance with claim 69 wherein the gene expression cassette is in an E1 antiparallel orientation.

- 72. An adenoviral vector in accordance with claim 69 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.
- 73. An adenoviral vector in accordance with claim 69 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

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- 74. An adenoviral vector in accordance with claim 69 which is at least partially deleted in E3.
 - 75. A cell comprising the adenoviral vector of claim 68.
- 76. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 68 into a cell line which expresses adenovirus E1 protein at complementing levels.
- 77. An HIV vaccine composition comprising purified adenovirus particles of claim 76.
 - 78. An HIV vaccine composition of claim 77 which comprises a physiologically acceptable carrier.
 - 79. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 68 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.
 - 80. A method according to claim 79 wherein the cell is a PER.C6[®] cell.

81. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 78.

- 82. A method according to claim 81 which further comprises

 administration to the individual a DNA plasmid vaccine, optionally administered with
 a biologically effective adjuvant, protein or other agent capable of increasing the
 immune response.
- 83. A method according to claim 82 wherein the DNA plasmid
 vaccine is administered to the individual prior to administration of an adenovirus
 vaccine.
 - 84. A method according to claim 81 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.
 - 85. A method according to claim 81 which comprises administering and readministering the adenovirus vaccine vector to the individual.
- recombinant, replication-defective adenovirus particles, wherein the adenovirus particles are harvested and purified from a cell line expressing adenovirus E1 protein, and wherein the particles are harvested subsequent to transfection of the cells with an adenoviral vector or vectors in accordance with claim 9; said vector(s) comprising a gene expression cassette or cassettes comprising nucleotide sequences encoding HIV proteins selected from the group consisting of:
 - a) gag, pol, and nef, expressed independently from three individual vectors;

 b) gag, pol, and nef, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;

- c) gag, pol, and nef, expressed via two vectors, one expressing a polnef fusion, and another expressing gag;
- d) gag, pol, and nef, expressed via two vectors, one expressing a gagpol fusion and another expressing nef;
- e) gag, pol and nef, expressed via two vectors, one expressing a nefgag fusion and another expressing pol;
- f) gag, pol, and nef, expressed via one vector expressing a gag-polnef fusion;
- g) gag and pol, expressed independently from two individual vectors;
- h) gag and pol, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- i) pol and nef, expressed independently from two individual vectors;
- j) pol and nef, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- k) nef and gag, expressed independently from two individual vectors;
- nef and gag, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- m) gag and pol, expressed via one vector expressing a gag-pol fusion;

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n) pol and nef, expressed via one vector expressing a pol-nef fusion;
 and

- o) nef and gag, expressed via one vector expressing a nef-gag fusion.
- 87. A multivalent adenovirus vaccine composition in accordance with claim 86 wherein the gag-pol fusion consists of SEQ ID NO: 39.
 - 88. A multivalent adenovirus vaccine composition in accordance with claim 86 wherein the fused sequences have the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences.
- 89. A multivalent adenovirus vaccine composition in accordance with

 10 claim 86 wherein the fused sequences have the encoding nucleic acid sequences

 operatively linked to a single promoter; and the encoding nucleic acid sequences

 operatively linked by an internal ribosome entry sequence ("IRES").

Original Adenovector Construct:

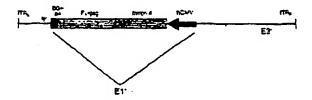


Figure 1: Original HIV-1 gag adenovector.

Sequence of the open reading frame for FL-gag (human codon optimized)

atgggtgctagggcttctgtgctgtctggtggtgagctggacaagtgggagaagatcaggctgaggcctggtgg caagaagaagtacaagctaaagcacattglgtgggcctccagggagctggagaggtttgctgtgaaccctggc cigciggagacticigagggigcaggcagatccigggcagctccagccctcccigcaaacaggctcigagg agctgaggicccigiacaacacagiggctacccigtacigtgigcaccagaagatigatgigaaggacaccaag gaggecciggagaagattgaggaggagcagaacaagtccaagaagaaggcccagcaggctgctgctggc acaggcaactccagccaggigtcccagaactaccccattgigcagaacctccagggccagatggtgcaccag gccatctcccccggacccigaatgcctgggtgaaggtggtggaggagaaggccttctcccctgaggtgatccc catgitctctgccctgtctgagggtgccacccccaggacctgaacaccatgctgaacacagtgggggggccatc aggctgccatgcagatgctgaaggagaccatcaatgaggaggctgctgagtgggacaggctgcatcctgtgc acgciggccccattgcccccggccagatgagggagcccaggggctctgacattgctggcaccacctccaccct ccaggagcagattggctggatgaccaaccaccccccatccctgtgggggaaatctacaagaggtggatcat ccigggccigaacaagatig:gaggatgtactcccccacctccatcciggacatcaggcagggccccaaggag ccttcagggactatgtggacaggttctacaagaccctgagggctgagcaggcctcccaggaggtgaagaact ggatgacagagaccctgctggtgcagaatgccaaccctgactgcaagaccatcctgaaggccctgggccctg gctgaggccatgtcccaggtgaccaactccgccaccatcatgatgcagaggggcaacttcaggaaccagag gaagacagtgaagtgcttcaactgtggcaaggtgggccacattgccaagaactgtagggcccccaggaaga ggcaeaatctggccctccacaagggcaggcctggcaacttcctccagtccaggcctgagcccacagcccct cccaaqaqiccticaggtttggggaggagaagaccaccccagccagaagcaggagcccattgacaagg ageigiaeceetggeeteetgaggieeetgttggeaaegaeceeteeteeaglaaaalaaageeegggea gat (SEQ ID NO: 29)

Figure 2

Old Transgene:



New Transgenes:

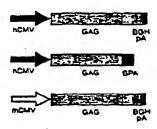


Figure 3: Diagrammatic representation of the original HIV-1 gag transgene and the series of new transgene constructions.

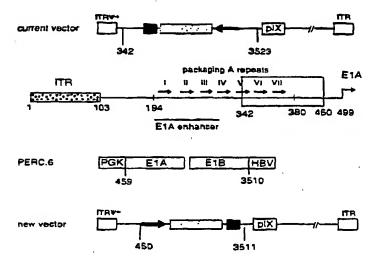


Figure 4: Modifications made to the current adenovector backbone in the generation of the new vector.

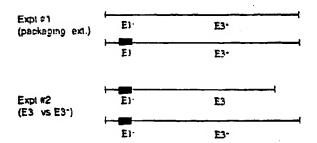


Figure 5: Virus mixing experiments to determine the effects of the addition made to the packaging signal region (Expt #1) and analysis of the effects of the E3 gene on viral growth (Expt. #2). The red bars denote the region of modifications made to the E1 deletion.



Figure 6: Autoradiograph of viral DNA analysis following viral mixing experiments (expts. #1 and #2) as detailed in the text.

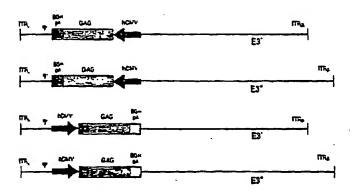


Figure 7A: hCMV-FLgag-bGHpA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

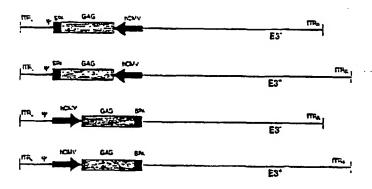


Figure 7B: hCMV-FLgag-SPA adenovectors constructed within the *MRK* backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

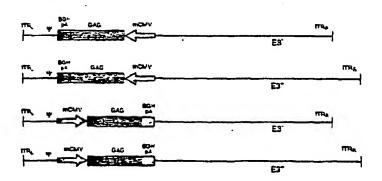


Figure 7C: mCMV-FLgag-bGHpA adenovectors constructed within the *MRK* backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

Plasmid mixing expt: (orientation)

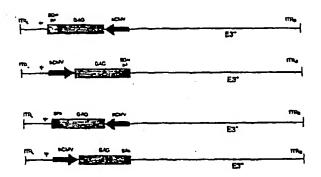


Figure 8A: Effect of transgene orientation

Plasmid Mixing expt: (poly A signal)

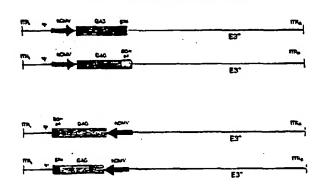


Figure 8B: Effect of polyadenylation signal

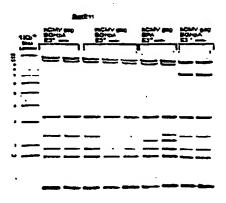


Figure 9: Viral DNA from the four Adgag candidates at P5, following BsfE11 digestion.

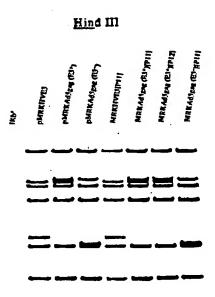


Figure 10: Viral DNA analysis of passage 11 and/or 12 of MRKHVE3, MRKAd5gag and MRKAd5gag(E3-).

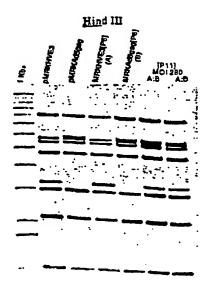


Figure 11: Viral DNA analysis (*Hind*IIII digestion) of passage 6 MRKHVE3 and MRKAd5gag used to initiate the viral competition study. Last two lanes are passage 11 analysis of duplicate passages of the competition study (each virus at MOI 280 vp).

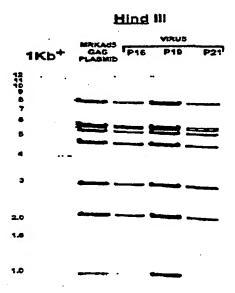
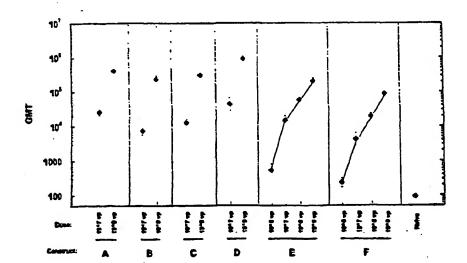


Figure 12: Viral DNA analysis by HindIII digestion on high passage numbers for MRKAd5gag in serum containing media with collections made at specified times. The first lane shows the 1 Kb DNA size marker. The other lanes represent pre-plasmid control (digested with Pac1 and HindIII), and MRKAd5gag virus continually passaged to P16, P19 and P21(serum containing media).

Figure . Serum anti-p24 Levels at 3 Wks post i.m. immunization of balb's mice (n=10) with Varying Doses of Several Adgag constructs: (A) MRKAd5gag (through passage 5): (B) MRKAd5 E3 bCMV-FLgag-bGHpA; (C) MRKAd5 E3 bCMV-FLgag-SPA; (D) MRKAd5 E3 mCMV-FLgag-bGHpA; (D) research Lot (293 cell-derived) of Ad5HIV-1gag; and (F) clinical lot (Ad5gagFN0001) of Ad5HIV-1gag. Reponed are the geometric mean titers (GMT) for each cohort.



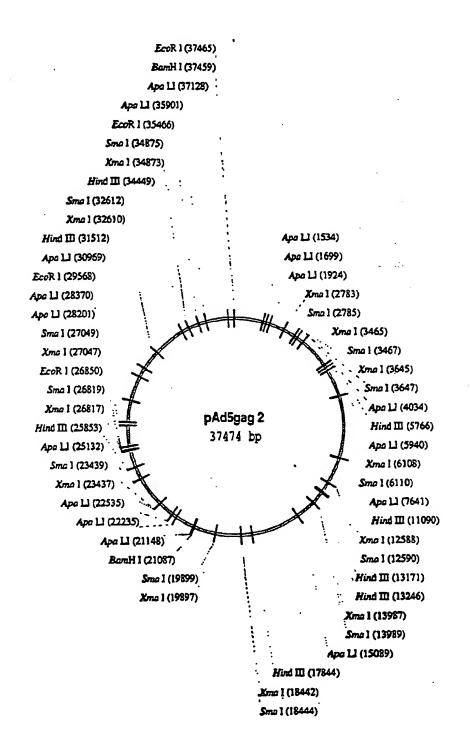


Figure 14

	-	į								Canada Broom
_	- TTCTTANTTA		TAATATACCT		THAN: CLANT	ATGATAATKIA	CONTRACTOR		ושבנא.שלאטר	College Waller
	MONTTANT	TICTACTACTT	ATTATATATA	ATAAAACCTA	ALTIC CHITA	TACTATTACT	הרנכאהכוכ	MACACITACA	כנינייינייניני	בשרכו בוניי
101	CATCOCCTOAC	GTAGTAGTGT	התבעההאהחה	TriA TriTiviCA	ACTITION NO.	AACACATGEA	ACK_TSACCIONT	CTCCCANANG	TOACOTTITI	מטונים אינויי.
	CCCCCCACTG		CCCCCTTCAC	ACTACAACGT	TUNCACCOCC	TYGYGTACAT	TOSCIOCOTA	CACCOTTATIC	ACTOCANANA	CCACACACA 1
201	CACTOTACACA	_	ATTTENDE	COTTTTACC	GRATHITICA	GTANATTYCG	CANTAMICGA	STANSAITTS	OCCATITITICO	CRESANANT
1	CCACATOTOT		TAMARKTATA	CCAMATCCG	CCTACAACAT	CATTFAAACC	COCATIVATOR	CATTCTAAAC	COSTINAAAGC	CICCCITTINIA
101	CANTABGACE		TGAATAATT	Tritritaria	ATATATA	ATATTTETET	AGRECCIATOS	GGACTITIGAC	CONTINCEN	GNOACTICIA C
•	CTTATTCTCC		ACTTATTAM	ACACAATGAG	TATELLEGICAT	TATAAACAAA	Tecendedec	CCTCAMCTO	GCAANTGCAC	CICIONOCO.
100	CAGGINGIPPIT	-	THECOUNT	CCOGGITCAAA	CHICKNICCTUT	TATTATTATA	פטינומכבמים	ATCCATTGCA	TACGFTGTAT	CCATATCAT
	OTCCACAAAA	_	ANACKACTOCAA	GGCCCAGTIT	CAMERITICANA	ATAATAATAT	כנומננססכפנ	TAGGTAACGT	ATCCAACATA	CCTATACTAT.
501	ATATOTACAT	TTATATTOOC	TCATGTCCAA	CATTACCOCC	ATCTFGACAT	PCATTATICA	CTACTTATTA	ATAGTAATCA	ATTACGGGGT	CATTAGETICA
	TATACATOTA	_	_	GTAATORCGG	TACAACTGTA	ACTAATAACT	GATCMTAAT	TATCATTAGE	TAATGCCCCA	CTANTCAAGF
601	TACCCCATAT	ATGGAGTTCC	GCGFTACATA	ACTITACOSTA	ANTRACCICAC	CTYNCTTHACC	שכככשכניים	CCCCOCCCAT	TOACCICAAT	AATCACCTAT
	ATCCCCTATA	_		TCAATCCCAT	TTACCGGGG	CACCCACTES	CGGGTTGCTG	GROCCOCOTA	ACTOCAGTTA	TTACTOCATA
701	GITCCCATAG	TAACGCCAAT	AGGOMETETE	CATTGACGIC	ANTOROGRAGA	GTATTTACK	TANACTYCCC	ACTTOOCAUT	ACATCANOTO	TATCATATY
	CANGGOTATC	-		OFANCTICING	TTACCCARRY	CATAMATISC	ATTTIGACIO	TOMOCOSTICA	TOTAOTICAC	ATAGTATACC
108	CAACTACOCC	_		CTAAAATKACC	CCCCTTAGGAT	TATGCCCAGT	ACATGACCTT	ATGGGACTIT	cctactrode	AGTACATOTA
	OFFICATOCOO	_			CCCGACCGTA	ATACCCAFTCA	TGTACTGGAA	TACCCTIGNA	GCATCAACCG	TCATCATAGAT
106	COTATTAGE	_	CCATCGTGAT	CCCCTTTTCC	CASTACATEA	ATISGRALITICO	ATAGGGGGTT	DACTCACOO	DATTTCCAND	TCTCCACCC.
	CCATAATCAG	•			OPCATVITAGE	TACCCCCACC	TATCOCCANA	CTCMGTGCCC	CTAAAGGTTC	MONGGINGRO
1001	ATTOROGEN			CAMATCANC	CAGACTITICS	AAAATGTCCT	ANCHACTECTS	CCCCATTGAC	GCAAATGGGC	COTACACOTA:
	TARCTICAGE			GTTTAGTTG	CCCTGAAAGG	TTTTACAGCA	THEFTEROCE	COCCUPANCITO	COLLITACCCO	CCATICKGCAC
1101	TACCOTOCOA	_		GTTTAGTGAA	CCGTCAGATC	GCCTATAMGAC	GCCATCCACG	CTGTTTTGAC	CTCCATAGAA	מאכאהכמוזיי
	ATOCCACCCT	_	TCGTCTCGAG	CANATICACTT	GCCAGTCTAG	COGNICACIO	COGTANGOTOC	GACAMACTO	CACCTATCTT	Crorexecur
							Roll			
1201	CONTRACTOR	CHCCCCGGCC	GOGAACGGTO	CATTOOMIC	COGNITICCC	GTGCCAAGAG	TCAGATICTAC	CATGGGTGCT	AGGCTTCTG	rocter; reg
	COCTACCTCC	_		CITACCTICC	GCCTAARAGG	CACCACITICAC	ACTICTAGATO	GTACCCACGA	TCCCGANGAC	ALGAL MGACE
1301	TYXTICACTO	_	AGAAGATCAG	OCTRIABORICT	COLUMBICATION	ACHACTACAA	CCTAAAGCAC	ATTOTOTOGO	CCTCCAGGGA	CACTIVITIANIS
! !	ACCACTCGAC	-	TCTTCTAGEC	CCACTCCCCA	CCACCGTTCT	TUTTONICATE	CGATTICGTG	TAACACACCC	COMCANTELLT	בפעררורור
1401	TTOCTOTOA	_			GCAGGCAGAT	CCTCOOCCAG	CTCCAGCCT	CCCTOCAAAC	AGGCTCTCAG	CHECKERET
	AAACGACACT	TOCOACCOGA	COACCTCTOO		CGTCCGTCTA	מנושככנמנוב	GAGGICGAGA	ואסטערפווופ	Treample of	44.144.04.004
1501	CCCTOTACAA	_	_	GIGIVICACCA	GANCATTCAT	CHTIANTIAL	CUNASTACK	REACCIETTE	TAACTCCTCC	TCOTCTION
	COCACATOTT	-	-		C11C1.1A	Cm. 1 1 cc 101	Charles Const	ATTORISADA	ACCTECAGO	CCAGATOOTU
1601	GTCCAARAG	-	-	TOTICACACACAC	ANCTICALATE	TYC AC ACACCT	CTTCATCOC	TANCACCITCT	TOGAGGICCC	GOTCTACCAC
	CAGGITCTIC	: דוככמונים	שבבניעו וישבו							

Figure ISA

PMRKAd5gag MER682

1701	CACCAGGCCA	TCTCCCCCC	GACCCTTAAT	מכנידיאיניה	ACITATIONS	CATACAACACC	THYTECHE	ACCITICATICCC	CATOTTCTCT	GCCCTGTCTO
	GTGGTCCGGT	AGAGGGGGGG	CTORONCTTA	CCRINCICKIACT	TYT/ACX:ACTT	כרדונידוכננאי	MGAGGGGAC	TCCACTAGOO	GTACAAGAGA	CONTINCAGAL
1001	AGG/TGCCAC TCCCACGGTG	CCCCCAROAC	CTCAACACTA	ACTIAL TATABLE	MITTER STREET	CANTACASCTS CTIAGTESCGAC	CCATCKACATA CAGTACTA	GCTGAAGGAG	ACCATCAATO TOOTAGTTAC	ARRANGCTOT TOCHTOTARE
1901	TOAOTOGGAC	AGGCTGCATC TCCGACGTAG	CHENTCACK	PRINCINCCAPT ACCCREMITAL	CHECKETTORICE	ACATERACISCA TYTEACTECOLT	CHOCAGINANC	ACTORCATION AGACTOTANC	CTORCACCAC	CTCCACCCI.
2001	CAGGAGCAGA	TTOCCTCOAT	GACT: NATING	מככבבנעוני	VIXXXXXXXX	ANTETACAAG	METRAINTCA	recreaseer	GAACAAGATT	OFTACTATES
	GICCICOTCE	ACCOACCTA	Creament	CAPARKTAGG	באניאניניניד	Tragatette	TCCACCTAGE	AGGACCCGGA	CTRATICTA	CACTCCTAGA
1017	TOAGGGGGTG	GAGGTAGGAC	CHUTACTUCE	ACCCCCCCCCTT	CCTCSCRIANG	ACCCTCATAC TCCCTCATAC	ACCTETECAA	GATGTTCTGG	GACTCCCGAC	TCURCOGAI
2201	CCAGGAGOTO	AAGAACTORA	TCACACACACA ACTVACTICTO	CCTACTRAGTO	CACANATGCA	ACCETTACTO	CANGACCATC	CTGAAGGCCC	TOGACCC TOC ACCCOGOACO	TOCCALICITY ACCOTOCOM
1062	CTCCTCTACT	TOACAGCTO ACTOTCOGAC	CCAGGAGATE	GRAGGRACTA	CASTRITUCES	CAMAGENACTA	GCTGAGTCCA CGACTCCGGT	TOTCCCAGGT	GACCAACTCC	GCCACCATC, CGGTGGTAGT
2401	TGATGCAGAG ACTACGTCTC	GOGGAACTEC	ACCITICGICT	GEANGACAGT	CTTCACGAAG	AACTVITICCA	ACCICCCCA	CATTGCCAAG	AACTGTAGGG TTGACATCCC	CCCCCAAAAA. GCACCTCCT !
2501	GAAGGGCTGC	TOGANOTOTO	OCANOGAGO OCTANOGAGO	CCACCAGATO	AACCIACTOCA	ATOAGAGACA	GOCCAACITIC	CTCCCCAAA	ACACCCCTC	CCACAAGACK:
2601	AGGCCTGGCA	ACTICCTECA	GTCCAGGCC7	CAGCCCACAG	CCCCTCCCCA	GOAGTCCTTC	ACOTTTCOOL	ACCACAACAC	CACCCCCAGC	CALLANGEAR
	TCCOCACCOT	TCAACCACOT	CACOTICOGA	CTCCCCTCTC	ССХСЛОТОСТ	CCTCAGGANG	TCCAACCCC	recrement	GTGGGGGTCG	memone.
2701	ACCCAPTUA	CAAGGAGCTG	TACCCCTTIO	CCTCCCTOAG	отссетит	GREANTRACE	CCTCCTCCCA	GTANNTAAA	ОСССООЛСАО	ATCTOCTOT:
	TCGGGTAACT	OFFICERCOAC	ATOCOGGACC	הנואממסאכדכ	CAGRICAAA	כנינידנאנדנינ	GOAGGAAGGT	CATITIATIT	COOCCCCIC	TNOACCACA
2801	CCTTCTAGT	OCCAOCCA TO	TOPPOPUTOC	CCCTCCCCC	TRECTICENT	GACCETORIAN	CCACCERCACT	CCACTOTOCT	ANGERTATA	ANTENCOAN
	COMMUNICAN	CONTRACTOR	אראוראארא	Charles	TO TO TO TO TO TO TO TO TO TO TO TO TO T		Company of the compan			Sirht
1062	Trocatcoca	TTOTCTOAGE	ADDIOTOTOATS	CTATTCHANG	CROSTRAGATO	RECARGACA	CCAACCAATA	CCATTOCCAA	GACAATAGCA	CCX:ATCTCTC
	AACGTAGCGT	AACAGACTCA	TCCACAGTAA Prof	GATAAGACCC	כככשכככבשכ	cccorccror	CGFTCCCCCF	CCTANCCCTT	CTOTTATOGE	CCCTACGACC
			Asc	- To						
3001	OGATOCOOTO CCTACOCCAC	GOCHCTATOO CCGAGATACC	CCOATCGCO CCCCATCAC	CCCCOTACTO	AAATGITGITG TITAGAGAGC	CATCHTAX: TTA	AGGETGGGAA	AGNATATATA TCTTATATAT	AGCTCCCCCA TCCACCCCCA	CITATOTAGE GANTACATEN
3101	AACATAGAC	TTTTGCAGCA AVACGTCGT	COCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	CCATY/MAGAC GCTACTC/FTG	CAACTCGTTT GTTSAGCAAA	CTACCTTOST	TTOTANGCTC ANCACTCGAG	ATATTTCACA TATAAACTOT	ACCIOCATOC	CCCCATAGGC
3201	CCCCACGCA	CARAMETERA	TROPA TY CAG ACCCGAGGTC	CATTGATTAGT GTAACTACCA	CCCCATACACA	ACCCCCCTTA	CTCTACTACC GAGATGATGG	TTGACCTACG	AGACCETOTC TCTGGCACAG	TOGANGGEGG ACCTTRACGGC

tique 15B

PMRKAd54Ag MERGB2

3301	AVECTETICAL	CARCCTCCCC	CCCCCCTTCA GCGGCGAAGT	האירואריהיאני בנאניינאניינולני	כאנונטאואאא.	GUTTANGAG	ACTIVACTORY	CHITCCTICAG	CCCACTAGCA	AACM:TGCAG TTYTE ACCITE	
3401	CTTCCCOTIC	ATCCIDECEGE TAGGERGEG	CATCACAGE	TCIACKER TCT ACTERCOMANA	TTTV: KCAUAA AAACCCIV:TT	TITETATHCTF AACCT/AAGAA	TOMOCCONTO ACTORGECCT	ACTTANTOTC TGANTTACAO	CAANGAGTCG	ACKITKITTRICA TCCIACIANI"	
3501	TCTCCCCAG AGACGCGCGTC	CACCAMAGAC	CCCTV:AAGGC GCGACTTCCG	TRUTHUCULT AAGGAAGARGA	CCCAATTECHO GEATTACHOC	TTTANAACAT AAATTTIKITA	TTTTTTTT	CCAGACTICTO OCTCTGAGAC	TTTOGATTTO	GATCAAGCAA CTAGTTCG1 r	
3601	GTOTCTFOCT	GICTITIATITE	ACCCCANANC	המתאכנוניה מכואהמכנוכה	ARGCCCCXXXA TCCGGGCCCT	הבאמנות: זריל מהדטרונינאמא	CONTROTTERA	CCCAGGACAC	TATTTTTTCC ATABABABOO	AGGAGGTGGT TCCTGCACCA	
3701	AAAGGTGACT	CTOCATOTIC	AGATACATGG TCTATGTACC	CCATARCICC	CAGATACTEC	THE AGGTANG ACCINCATED	Pell ACCACTVICAG TOCTGACGTC	AOCTTCATGC TCGAAGTACG	TOCOCOCOTOO ACOCCCCACC	ACAACATTA	
3801	GATCCABTCG CTAGGTCAGC	TAGCAGGAGC	CCACCOTCOTC	CACGGATTITE	ATCHUTTICA TACAMMAGT	GTAGCAAGCT CATCGTTCGA	CTAACGGTCC	GOCAGGCCCT	TCOTOTANOT	GTTTACAAN: CAANINGTTTC	
3901	COSTINGER	CCCTACCCAC	CATACCTORYO	GATATRAGAT CTATACTCTA	GCATCTTAGA	CHSTATTTIT	ACCANCEGAT	TUTTCCCAGE ACAAGGGTCG	CATATCCCTC	CORRESTIVIA RECECTAMENT	
4001	TOTTOTOCAG ACAACACOTC	AACCACCACC	ACAGTOTATC TGTCACATAG	CCCACCTCTAA	CCCTTTANAC	TCATGTAGCT	TAGANGGAMA	TCCCTCGAAG ACGCACCTTC	NACTTOGAGA TTGAACCTCT	CCCCTTGTY	
4101	ACCTCCAAGA	TTTTCCATCC AAAAGGTACG	ATTCCTCCAT	AATGATOGGA TTACTACCGT	ATTATACCAC TANCECOGGIG	CCCGCCGCG	CHIBOCOMA	ATATTTCTOG TATAAAGACC	CHACACTAAC	OTCATIVETED CAGITATECAN	
4201	TOTTCCAGGA	TOAGATEGIC	ATACACATT TATCCACTAA	TTTACAAAGC AAATGTTTCG	GCCCCCCCCTC	CCACOGICIO	TCCCCTATAL	TOGITCCATC ACCAAGO (COGCCCAGGG	GCGTACTTA COCATCAA'n:	
4301	CCTCACACAT	TTGCATTTCC AACGTAAAGG	CACACTTHGA	GTTCAGATOG CAAGTCTACC	CCCCTAGTAC CCCCTAGTAC	TCTACCTGCG AGATGGACGC	CCCCTACTT	GAZAACOSTE	TCCOGOCTAG AGRICCCCATC	OCCIOCITATION CCCICICATION PSI	
4401	CTCGGAAGAA	AOCAGGTTCC TCGTCCAAGG	TOACCACCTO ACTCGTCGAC		GENETANCES CARICYOSTICS GCTSIANTEGE CITCOCCACE	GCCCGTAAAT	CACACCTATT GTGTGGATAA	ACCOSCIGCA	ACTOOTAGIT TGACCATCAA	AAGAGACTI:	
4501	CAGCTGCCGT	CATCCCTGAG GTAGGGACTC	CAGOTAGACE OPECECECEGG	ACTICATION TOANGCANTE	GCATGTCCCT CGTACAGAIA	GACTECATATA	ANAAROGACT SAIN	CCAAATCCGC GGTTTAGGCG	CAGAAGGCGC	TCCCCCATCCA AGCCGCCGCT	
4601	OCCUPACOCAG CCCTATCOTC	AND AND AND AND AND AND AND AND AND AND	GAAGCAAAGT	THITCANCYO AAAAGTHECC	PPTGAGAGGG	PUCKETTAG NPRKETATATE				CCAGGCGGTC	
4701	CCACACCTCO	OTCACCTOCT CAGTGGACGA	CTACCACATC	TCGATCCARC ACCTACCTCG	ATATCTCCTC TATAGAGGAN	CANACACAC				CCCACGAGGA	
4801	CCAGACGGGC	CAGGGTCATO	AGAMAGGTCSC	הכמכנות היא	CCTCTTTACC	CATCAIACCC	TCACCACTT C	CCCCACCACA (מסכנכמענטב	הכנאניבנים זיי הכנאניבנים זיי	

figure 15c

рмпклабара мел682.

4901	amacactro	AGGCTOGFCC	TECHOTICE	GAARSTA-TVIC	COGINETINGE	בנשטישטשב	COCCAONTAG	CATTTOACCA	TCCTCTCATA	פאבבאטיניניב	
	CCACCCCAAC	TCCGACCAGG	ACCIACCACCIA	CTREACTIANTS	CKCACAAACC	מאנעאנענענע	CCURRENCATO	GTANACTIOGF	ACCACAGTAT	CARROTCOORN	
2001	recococcot		OCCCAGETTE	CCCTTYX:ALT	אנשונינו נימכא	נגועטשטטטענעט	TYCACACTTT		GAGCTHRAGGC	OCTIAGADATIA	
	AGGCGCCGCA	CCCCCCAACCC	CCCOTCGAAC	CHICANCUTCE	TCCGCCCT	(# TYTCTCGTC	ACCITCIANA	ACTCCCCCAT	CTCGAACCCO	COUNCTITAT	
5101	CCGATTCCGG	GONCHAGISCA	Tecaecae	AGGCCCCCCC	GALGGETTE	CATTCCACGA	CHICANSTANG	CTCTOCCCOF	TCCCCCTCAA	MARICAGGIT	
	OCCTAAOOCC	CCTCATCCGT	AGGCGCGGCG	recoggecer	CTRCCAGAGG	ataacetect	COORCCACTE	GAGACCOGCA	AGCCCCAGITY TIT	TTTGGTCCA?	
5201	TCCCCATOC	TITTICATOC	GTTTCTTACC	TERROTTICE	ATVINACOCK	התכמכות	COTCACCAAA	ACCEPTE	TOTOCCCOTA TACACIACTY	TACATACTT	
	ACCOCCTACO	AAAAACTACO					CCACTGCTTT		ACAGGGGCAT ATCTCTGAM	ATCTCTOAM:	
		Khol		٠						•	
5301	AGAGGCCTOF	0	TOTTCCTACGG	Techecited	ATAGAMACTC	GRACCACTCT	CACACAAAGO	CYCOCOTICA	OCCCAGCACO	AACKINCKTA	
	TCTCCGGACA	OGAGCTCGCC	ACANGGERECE	ARCARCACCA	TATCTITICAG	CCTOSTIGAGA	CTCTOTTTCC	OAGCOCAGCE	ccoorcoroc	TYCCTCCGAT	
5401	ACTOCOCADOO	OTACCOOTCO	THITCCACTA	GOOGGITCCAC	TUTCHCANG	MUSTCAAGAC	ACATGTCGCC	CTCTTCGGCA	TCAAGGAAGG	TOATTOSTT	
	TCACCCTCCC	CATCOCCAGE	AACACCOTCAT	сссссуддья	NACTANGENCE	CACACTTCTO	TOTACARCOG	GAGAAGCCGF	AGTECCTICC	ACTANCCANA	
5501	GTAGGTGTAG	OCCACOTOAC	COCONGINC	TEANGGOODS	CTATAAAACG	GACTGGGGGC	ACCOMPANDED	TCACTCTCT	CCCCATCCCT	GTCTGCGAGG	
	CATCCACATC	COGRACACTIO	OCCCACAAGG	ACTICCCCCC	GATATITICC	CCCACCCCCC	CCCAARCAGG	ACTORORGAN	OCCUTAGEDA	CNGACCETEC	
5601	OCCAGCION	GOOGTGAGTA	CTCCCTCTOA	AAAGCGGGCA	TOWCTTOTAGE	GCTAAGATTG	TCAGTTTCCA	AAAACGAGGA	DOATTIGATA	TEACHERY	
	COGITEGACAA	CCCCACTCAT	GAGGGAGACT	THEGECEGI	ACTONAGACG	COATTCTAAC	ACTCANAGGT	THEFTECT	CCTAMACTAT	ANCHOGACCT	
							- Fire III				
5701	CCGCGGTGAT	OCCUPATOROD	occrimana braccecar	CCANCICATIC	ACHANAGACA	AGAMAGACA ATCTTTTAT	TOTCAACCTT	GETEGEANAC	GACCCGTAGA	CRANCIPPIGN	
	DOCOCCACTA	COGNANCICC	CACCOCCOTA	OCTAGACCAG	TCTTTTCTGT	TAGANNAACA	ACAGTTCGNA	CCACCOTTTG	CTGGGCATCT	CCCOCAACCT	
					Pyth						
5801	CACCAACTTO	OCCUATOGACC	ослосоотто	OFFITTION	CCATORCCCC	ACTECT TODG	CGCGATGTTT		ATTCGCGCGC	AMCCCACCCA.	
	OTCOTTOAAC	COCTACCTICS	CONCCCARAC	CAMANCAGE	CCTAGCCGCG	CCAGGAAACCG	CCCCTACAAA	TCGACOTGCA	TANGCOCOCO	דו מכמוסמכיי	
5901	CATTCOOGA	AGACOOTGOT	OCCUTCGTC0	DOCACCARST	CCACTICICCA	ACCIRCOSTTO	TGCAGGGTGA	CANCOTCAAC	octooroct	ACCINCTICCO	
	GENAGOCOTT	TCTGCCACCA	COCCAGCAGC	CCGTOGTCCA	CONTOCOCOST	TOTOCOCONC	ACCITCCACT	OTTCCAGTTG (COACCACCOA	TOCAGAGG	
6001	UTACOCOCTC	GITGGICCAG	CAGAGGCGGC	COCCCTITION	CGAGCAGAAT	GALCATAGAG	GOTCTAGGTO	concreoree		CCTCCACCAT	
	CATCCGCGAG	CANCCAGGTC	Grenecisco	GCGTAGAALTIC	CCTCOTCTTA	CUCCUALCE	CCAGATCGAC	CCMONOCAGO	CCCCCAGAC	GCAGATACCA	
6101	AMAGACCCCG	OCCADENDO	OCCOUNTCOAL	GTASTCTATC	THECATECTE	GCAACTCTAG	CRECTRICTEC	CATGCGCGG	COCCANDECEC	CCCCTCGTAT	
	TTICTOGGGC	CCONCONCO	COCOCAGCTT	CATCAGATAG	ANCOTAGGAA	COTTCAGATE	GCCASACGACG	GTACGCGCCC (OCCUPACION (COCCAOCATA	
6201	COUNTRACTO	GOOGACCCCA	TOCCATOCOG	TRECTTACCO	CHINCHECTA	CATCCCCCAA	ATCTCTTANA	CCTAGAGGGG	CTCTCTGAGT	APPECAAGAT	
	CCCMCTCAC	CCCCTOOOOT	ACCUTACCC	ACCENCACIOC	GCCTCCGCAT	GTACGGCGTT	TACAGCATT	GCATICTICCCC	GAGAGACTCA	TAAGGTTCTA	
6301	ATCTACCOTA	OCATCTTCCA	CCCCCATATAC	TEXACESCAC	GENATIONAL	Amtomoca	MASSAGGGAG		-	TACOCOCOCY	
	TACATCCCAT	COTAGANOOT	OCCCCTACO	ACCOCOCOTO	CATTACK:ATA	TCAAGCACGC	recereacte	CTCCAGCCC+ (OCCITICANCO !	ATTRECEDITEC	
6401	CTOCTCTOCT	COGRAGACTA	TETECCTICAL	CATCACCATGE	GACTERIONE	ATATOMITAN				DANACTTACI:	
	GACGAGACGA	OCCURCION	AGACGGACTT	CTACCGTACA	CTCAACCTAC	TATACCAACC	TOCCACCTTC	TOCAACTICG !	ACCOCACACA	באראגארטי:	

Figure 150

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THEACHTETA CARECOANTA STECAURITY TECTTOATOR ACTIONARIA CECACOTENT CAGSTECCAA AGGMETACT	GRICHTICA GIACICTIOS AICESABACE CONCRICI" CCAGNANGOT CATGAGAACE TAGCCTITIGS GUARICHIGM:	THEFACHEST ACCOCATATO COTOCOGODO CTROCOSIANO AMENTACION PODGOGOTATO GOACOCOCOO GAAGOCOTIVIE	TCAGTGTCGT CGCATCCTCC CTGCTCCCAG ACCAAAAGT AHTVCACAGCA GCGTAGGCGG GACGAGGGTC TCGTTTTTCA	THYCCCCCC ACCENTANG THECHTEN TECHNORY: ANISOCCC TCCCTATTC ANCOCACACT ACCENTIC:	GTHUNTUTTO TOOCCCACAA TOTAANOTTC CANGANGCGT CAACTACAAC ACCGGGGTT ACATTFICAAG OTTCTTY,FCT;	CYTAGONOT GENETIANAG GEOCCAGTET GCAAGATEAG GACTOGOGA CAAACTTIC COGGICAAA COTICTACTI:	GANNOTICCT ANACTOGOGA CCTATOGOCA TTTTTICTOR CTTTICONGA TTTOACCOCT GOATACCOOT ANAANGACC	TAGENTICOC OCOGCADICA CTAGAGOCTO ATCTCCOCO ATCCANAGOS COCCOTEAST GATCTCCOAG TAGAGGGGG	GRENETAGAT COTAGORAG ANAGAGACOC TEGITOCOAA CAGAGATGTA OCATECACTO TITETETOGG AGECACOET	TOTICOTRIAAN OTAGAAGTCC CTGCGACGGG CCGAACACTC ACACCACTTT CATCTTCAGG GACGCTGCCC GACTTGTGAAA	CACCAGETTO ACCTOACGAC COCOCACANO DAACAGAGT GTOCTCCAAC TOGACTOCTO ACCOCATOTTC CTICGTCTCA KM61		TEMENACATE GEGERATES GRACTISTECA TRATICIODAS ACTUTISTAS EGGERETACE CICCIACATO ACENGACETE	OCCIONACT AGATICAGGT GATACCTAAT TTICAMIGAC COCCIONA TOTAGGTICA CTATGGATTA AAGGTICOCO	מאידאריניזוא מיאאריניאבט פטכאסאפטב פסכאסאנין זכנידופאוזי
TCACCCACCITY, GRACKETHIANG 1 ACTIVATINGHIA CAGACANTRAG A	CANCICCINT TINAMAGES	POSTAGOCOC ASKATOCOTE 1 ACCATOCOTA TOCTAGOSAA 1	TEACHTACTE CTATTEGAG 1	CACATCETTY: AMENGTATET 1 CTOTAGGAAC TICTCATAGA 1	ARCACANTET COTYANAMEC O	AGGINGACITE INCACIOGAG (TECACIOCAG ANGRECETE	TAGENTAGE ANGRESIEGE CATEGITANAGE TECAGEAGES	TCCCATCTAN GGTTCGCGGC 1	AGGEETEENT CEANGTATAG (ATTEGNATAG TROCTATTGA 1 TANCCTCCTC ACCGATAACT A	TREMETAKET GTACATECTO C ACOTECECIA CATOTAGGAE C	CTICTIACTIC CACTACTIBE C	GRIGGERIC CERNECTTON 1 CREEKEGGEN GRETEGANET A	ACCTCOCATA GACKASTICAG IXOCOCAFGCT TOCACCATAT CTACCCAGTC CCOCGCCCGA	
OTACCIACITEO CACACETTAT 1 CATECITEARE GIGHEGAACA A	THITTHEC ACAGETCIES O	TOTAGAACTO OTTGACOCKC 1	GOTOTCCCTG ACCATGACTT 1	GGATTTGGCA CAGCGANGGT CCCTANACCGT CCCTACCTTCCA C	HOFFANTAC CYCGGCGCCI I	CANTITITA AGIICCICGI I	CACTOCCOTT	ANGGOTOGO	GOGCACOMO INCITICCOM /	AACTOGATCT CCCGCCACCA /	OFFICE CATA CTAGGARTAG 1 CACGCGTCAT GACCGTCGCC 1	TESCENSITY OCCUSINGS OF ACCESCENTS CONCERCED OF	AGATOTOCOC TCTACAGGCG	COCOTACTIC CTTSCACITITY /	PRECENTATION (RECEIVENTE CECVICATE)
CCOTCACOCA COAAGGAGGC G'	•	CGAACOGTAA GAGGCTAGGA TA	GAGGIOTODO TGAGGGGAAA G		PEGGAACOOF ARCETTOCCA	CCCTACCCCT TCATCGAAGG C	CHOCHTACTC	THEAAGOTAA	CCACCATGAA	GATGCGAGCC GATCGGGAAG A. CTACGCTCGG CTAGCCTTC T	TTOTAAAAC	OCCANTITON OCCCCTCOCC TO	COCCOCOCO	CTCCCGCGC OTCAGGTCAG G GAGGGCGCCG CAGTCCAGTC C	S TANTANTA CERTANISAT G
6501	6601	6701	6801	6901	7001	7101	7201	1301	7401	7501	7601	7701	7801	1961	1000

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8101	ATOCATCTAA	MOCOGOTCAC	GCCCCCCCACC	ררנינינים: אואיד	ACTION	כננאניענינינ	CCCCACACACIO	GOCAGGGCA	CONCERNO	פניחכוסוסמאנ
	TACGTAGATT	TTCGCCACTO			TECTTECTA	GENERAL	מאניניוכיוניניכי	ccorccccof	GCAGCCGCGG	いいつつじいいつい
0201	ASSAGETAGE	OCTOCOCCC		ניכנואאכבינינא			TOANTOTOG	CCCTCTCCCT	CAACACCACG	ממכנוסייוניא התמשאריאריו
	TCCTCGACCA	CONCOCOCOC	-	CGCTTCFLCT	(2,314.12,134.	CAM. JAMANA		Coorning	20120111	
8301	CCTTGANCCT	CAAAGAGAGT		CAATTICAGE	CHUCHTICACIO	والمراجعات والمراج		CTOCACOTO	CCTCAGTTGT	CHICATAGGG
	CGRACTTUCA	כדדוכוכא	AGCIGICITA	GTTALMGCCA	יאר אאר ווינר זיין	מין ניואישכרני	CCTFTTMGMG	STATE OF THE PARTY	מפער וכשירש	
8401	GATCTCOCC	ATGAACTOCT	CUATCTCTTC		CHICEMOGRACIA TETRECIOCITIC	נושוניונישבווכ	CACOOTICCG	GCGAGGTCGT	TOGRANTOCO	CCX.CATGAC
	CTAGAGCCGG	TACTTGACGA	OCTAGACAAG	GARMACCTAT	ACATICGCAG	GCCCAACCGAG	CTYCCACCCC	COCTCCAGCA	ACCTITACGC	CCCFFFACTIC":
8501	TOCCHONGO	COTTOACICC	Technome	CAGACGCAGC	TETTAGACCIAC	GCCCCTTCG	GCATCGCRAG	COCCECATOAC	CACCTOCOCO	ACMTTGACE F
	ACOUNTINCO	GCAACTCCGG	AGGGAGCAAG	grenacacen	ACAINCINGGING	CLYSCHYSMAN	CCTAGCCCCC	OCCCCTACTO	OTGGACGCGC	TCTAACTCO.
8601	CCACOPOCCO	GGCGAAGACG	OGCGAAGACO OCOTAGITIC	GCACGCTC	AAAGAGGTAG	THEACHERS	naccianting	TYCHOCCACO	ANGANGTACA	TAMCCCARCY:
	OCTUCACOCC	CCGCTTCTGC	CCCATCAAAG	CONCEGEDAC	TITCTCCATC	MCTCCCACC	ACCCCCACAC	AAGACGGTGC	TECHTCATOR	ATTOOOTC():
		T.	Foofiv							
8701	TCCCMCGTG		DATTECTION TATCCCCCAA	OCCUTCANOG	CGCTCCATION	CCTYTTAGAA	GTCCACGGCG	ANCIFICAAAA	ACTORGAGIT	OCCICCOCCOAC
	ACCOPTICCAC	CTANGCAACT	CTRACCAACT ATAGGGGGTT	CCGCAGTTCC	GCGAGGTACC	CANGCATCTT	CAGGTGCCGC	TICANCTITI	TOACCCTCAA	כמכמנספכוני
8801	ACCOUNTANCT	CCTCCTCCAG	AAGACGGATG	ADCITECTION	CAGTOTCOCG	CACCTURENC	TCANAGGCTA	CAGGGGGCCTC	TICHETICE	TCAATCTC!:
	TOCCAATIOA	COMOCAROCIC				GTCGAOCGCG	AGTITICCGAT	GTCCCCCGGAG	AACAAGAAGA	ACTTAGAGGA
									Brita	1
1068	CTTCCATAG	OCCUCCCT	refrement	CTOCCOCCC	TYSTATICACK	GCCACACGC		GCOCACCOGO		CAMBEGETE
	DANGGTATTC	CCOGAGGGGA	ADMGMGM	פעיבפכבפכב	ACCCCCTCCC	CCCTOTOCCG	ככשבונוכרוככ	COCOLOGOCC	TCCGCCAGCT	פאבעמעמאים
9001	GATCATCTCC	CCCCCCCCAC	OCCCATION	CTCGGTTACG	מבטנינישיבנים	Triciacocod	GCACAGTTOO	AAGACGCCGC	CCORCATIGAC	CCCASTTATES
	CTAGTAGAGG	OCCOCCOCTO	CCCCCTACCA		CGCGCCCATCA	AGAGGGCCCC	CCCCTCAACC	TTCTGCGGCG GCCAGTACAG	OCCUPTACAD	GCCCAATACT :
1016	GTTOOCOOO	OCCHOCCATO	COCCAGGGAT	ACCOCCTAA	CGATTICATET	CARCANTINE	TUTOTAGGTA	CICCOCCOCC	GAOGGACCTO	ACCCARTCY
	CANCCOCCC	CCCACOGTAC	OCCOTOCOTA	TOCCGCGATT	GCTACGTAGA	GITGITAACA	ACACATCCAT	Омассавсав	CICCCTOGAC	Tegeteaga
			XOros							
9201	CATCUACCOG	ATCOGAMAC	CTCTCGAMA	ACCIONTETAA				COTOBCOORC	OGCADCGGGC	OCCCOTCOTA:
	GTAGCTGGCC	TAGCCTITIO	GAGAGCTCTT	TECCENGATE	GGTCAGTGTC	ACCCTACCAT	CCCACTCGTG	GCACCGCCCG	CCCTCGCCCG	CCOCCAGCCT
		•					Snil			
9301	GIROTICIO	OCOGAGOTOC	TOCTGATGAT	GTAATTAANG	TACKACASTRUT	TUAGACTGCG	CATOCHICAC	AGAAGCACCA	INTECETIONS	recencence
	CARCAMAGAC	COCCTCCACO	ACGACTACTA	CATTANTITIC	ATCITATORD	ACTICTOCCOC	CTACCARCTO	TCTTCGTGGT	ACAGGAACCC	ARCCCASACTO
9401	TOAATOCOCA	occoorcoc	CATTRCCCCAG	OCTICONTA	GACATICATION	CACATRICATIVE		GCATGAGCCT	TICTACCOOC	ACTICITICE
	ACTTACOCOT	CCCCCYCCC	OTACOCOCIT C	CGAAGCAAAA	CTRITAGECOC	CHECANAAAC	ATCATCAGAA	COTACTCOCA	AACATOCCCO	TCANGNAGN.
9501	כתכבשכנת	TTOTECTUCA	TCTCTTCCAT	CTATCACTOC	تعدمتنون	CACTTINATE			CCCATOCOTO	TOACCCCOAN
	CACCANCON	AACAGGACGT	AGAGAACCTA	GATMXGACG	כניאכניאכניטכ	CICANOLIS	CATCCACCC		CACCTACCACAC	ACTRODUCT F
9601	CKCCCCATC	COCTGAACCA	CARRETACOTE	CHARCIACOACO	CCACTATION	ATATCACCTO	CTICACCTOC	GTCAGATTAG	ACTORNAMETIC	APECATION OF
 	COCCACTAG	CCGACTICGT	CCCCATCCAG		GCCACCCGAT	TATACCCGCAC	CACCTOCACO	CACTCCCATC	TGACCTTCAG	TAGGTACAGG

Figure 15F

	501	ü: Fe	" L L "			z = 5 5 5 5
TCCCTIGTACC ACCCACATURE CCCCCTTAGACC	GCCGGCGCGC COGCCGCCCCCCCCCCCCCCCCCCCCCC	CCCTGCTGPT GCCTGCTGPT GAGTGCTCCT CTCACGAGGA	CCTAATTCAL CGTAATTCAL GGGGGTTTCT CCCCCAND	GCTCTACGC GCGCTTCACGC GCGCTTCACG CCCCAACTTCACG	a coccanana A occananana T cogoctere C craconacco C coccanacco A coccanacco Handiii	CCTTTANCAA CCAAATTGTT TAOCAAGCGG ATCGTTCGGG CCCTGGGTGG GCGACCGACG
CTGCCARGAGC GACCCTCTCG GGCGACGGCT CCGCCGCCGA	TCCAGGTGNT AGGTCCACTA CTGGCCGGTC GACCGGCCAG	CCATACATOR CCATAGTACC GACAACGORO CTGTTROCCCC	GAAAGCGAAA CTTTCGCTTT TGCGGCGAAC ACGCCGCTTG	CCGTCCTCCC GCCACCACCCC GCCGACATCC CCGCTCTAGG	COGETHAGARA COCCERTERY GOCGANICCTE GEGETACAN TECETETECT COGGETECT TOAGECEGAC GEGEGAALCE ACTEGGGCTO COCCETTOGC ACTEGGGCTO COCCETTOGC	THECANAM CETTINGAN ANGITETT CRANTTITT ANACCENA THOCHREGG THTOGGITE ATCOTECONG GOCCONGOGG COCTOGGROC COGCOTECCO
CCACTCACCCOS CCACTCACCC CAAAAAGTGC GTTTTTCACG	TACCTOGACA ATOGACCTGT TCGGACGCT ACCCCTGCGA	AATTCGCAAG TTAAGCGTTC TGCGACGTCA ACGCTGCAGT	CCAATCCOAC CCTGCCGGAC CCTGCCGGAC	CAGATOCATC OTCTACGTAG CGTCAGGAGG GCAGTCCTCC	GGGCCTGGGG CCCGGACCGC CGCGACCGCG GCGCTGGCCGC AGGAGGACTT TCCTCCTGAA	GGAGATTAAC CCTCTAATTG GCGCTGGAGC CCCCGACCTCG ACATAGTAGA TGTATCATCT
		CTCCTIVGATA GACCACCTAT AACCCAGGTO TYCCGAGGTO	CMXCSTANGC GREGCATTCG GAGTCTCGGA CTCAGAGCCT	TTGCTTTTCC AACGAAAAGG CCTCCTACCG GGAGGATTGCC	AGGAGGGA TYCTECCGET GAACCTGTT CTTGGAGAAA TTGCTGGGGG	CCGTGANCCA CTTTTTANGC GAAAGATTCG GCGTCGTAAG
אאכיהיא דדוייניד הראיזיי דראיזיי	CCGCTACTAT ACIOTEMIANTO CCGCTACTAT AGGICATCTAC CCACAAAAAG TGCTCCATTAG GCCGTTATTTC ACTAGGTACC	CTHACCORDGE GRAGOCIACEA CCCCCHINICO GREECACAGC	TRANCE GROUND ANCES GROUND COCC GROUND GROWN GROUND GROWN GR	AGCCCCTTTT TCGGGGANA ACCTCCCTT	CTREACTTREA GACCTREAALC TOCCIRCTATA ACCISCOCCT TREACGAGEGO ACCISCOCCCC	
AGTINGGCAT TCAACCONTA TCAACTCONTA GAAAGTCON	CCCCTTAN AGGITGIAN CCCCTTAN AGGITGIAN CCANTCTAN AGGITGIAN CCANTGTAN AGGITGIAN	AGCOSTACTA TCGCCCGTGA CGGTTACCGC GCCAATGACG	TTTTTTTTCCAC AAAACCGTTG AATTCACAGAA	AACAGOGACO TTESTCCTGC CATGGAGGGG	CCCCCACTAC GGCCGTATO GACCTGTACG CTCCGCATGC ATGGCCTGAA TACCGGACTT	CCATTGGGTT GGACTGGTTG GGACTWG CCTGACTWG ACANTGACTG TGTTGCTGGG
CHARTECTAL METROCCAT CHARTECTU TCAMETECTION CETAMETECTT FETAMETECTIC GCATCAMETA COTTEMBERS	CCCCTTAG AGGTTGTATA CCCCTTAGA AGGTTGTATT CCATTCTAGA TGTTGCGCAG GCCAAGGTCT ACAACGCGTC	AGAGCCHTTA TCTCGGACAT GTGATCCATG	GCGCTAGCTT CCCGATCGAA CCAAGGCTTG	TTCCTCCGGA AAGGAGTCCT CAGCGGCAGA GTCGCCGTCT	OGCYCCGGGC CCTACGGCGT TUATAGGCGT ACTATGGCGGA GAGCTGCGGG CTCCAACGGCGG	CCGCCGACCT GGTGGCTATA GGTGGCTATA CCACCGATAT CACAGCATAT GACAGCACCC
OGTATOCOCC CONSTIGATS HIGTANATIKE CCATACOCOGC COCCAACTAC (INCATTERACE) STAG GTAGCCCTC GAGTCAAATA CCTTATTOCTTT CATTCGGGAG CTCAGTTTAT GCATCAGTCAA HAII	GOOCTCCPAG CCCGAGGCCC OTCACGAAGG	CACOTTTCC GCCGTCCTCC GCCGTCCTCC	OCCOCTOCT CCCCCOACGA CCGCTAATTTT	CGCTTGCAAA GCGAACGTTT AGAGCAAGAG TCTCCTTCTC	GAACCCCCGC CTTGGGGACG AGCTGAACTTCGC TCGACTTCGC CGCACGCGGG	CACGTGGCAG GTGCACGGCC CGCCACGAGGA GCGCGCTCCT TATAGTTGCAG ATATCACGTC
CCATACCCC CONSTIGNTS CCATACCCCC CONSTIGNTS CASTACCCCC CACTACAAATA CASTACCCCTC CACTACAATA CASTACCCCTC CACTACAATA CASTACCCCTC CASTACCCCTC CASTACCCCTC CASTACCCCTC CASTACCCCTC CASTACCCCTC CASTACCCCTC CASTACCCCTC CASTACCCCTC CASTACCCCTC CASTACCCCTC CASTACCCCC CASTACCCCTC CASTACCCTC CASTACCTC ASTACCT CASTA	ARBOTODOCO TCCCACCOOC COCOCOCATY GCOCOCCTTY Rbs1	OCTCTAGACC CGAGATCTOG CCCGTATCCG	TTCCAGGGGC AAGGTCCGCG TGTAGCCGGA	TOCANGACCC ACOTTCTGGG AGCAGCGGCA TCGTCGCCGT	ACCACTAATO CCANGGOTOC GGTTCCACG GAAAGTTCCA CTTTCAAGGT	COCOCOCOCOCA OCOCOCOCOCA ACCCTIVATO TOCOMACACC ACCTUTICCT TCGACAAGGA
ACAAAGCGGT TGTTTCGCCA TGAGACGCGA ACTCTGCGCT	GOCCCAOCOT CCCCGTCGCA OTCCTCGAGG CACCACCTCC	AATCUTTOAC TTAOCAACTO GOOTTCOAGC	THOOCTICE AACCGAAGG GCTCGCTCCC	CTCCCCOTCA GAGGGGCAOT CCCCCTCCTC GGGGGAGGAG	CCCCCACA CCCCTCCTCT TUACCGCAC ACTCCCCTG ATACCGCATC	CCTANTCAGG CCTACTGGG CCACGTGGG CCTCATGGCG CTCATGGCGC GAGTACCGCG
9701	10001	10101	10301	10501	10701 10801 10901	11101

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11301	TCCAPTTCAT	AMCATCCTO	CAGAGEATING		CHILLYNY, ILLU				TATTICCATOL		
	AGCTANACTA	TITOTACGAC	GICICOTATO	ACCACATOCA	ניניון איז איז איני				NI MOOI MO		
11401	CANTITAC	CCCCCCAAGA	TATACCATAC	CCCTTACCTT	CULININIACA	ACCIACIOTANA		_	CCATCCCCCT	GAAGRETTECT	
	GETCAAATO	COGGCGTTCT	ATATCGTATO	GOGNATOCAA	CASSTANT TRIT	TITLYCATT	CINCIDENCE	ANGATOTACO	COTACCOCOA	כבבוניבעכני י	
11501	ACCENCIANGERS	ACCACCTOGO	COTITATOR	MCCMACCA	TUCK ANOTE	στικοινίστο	איכבטטטעני	_	CONCCOCUAG	CTYSATISCACA	
	TOTAACTOR	TOCTODACCC	GCAAATAACG	Tractedest	ACKSTICT TRICKS	CCALTECTICAC	איניפככנוכנים	COCTCGAOTC	GCTGGCCACTC	CACTACOTATE	
10000	COMPANY		CHERTATION	CONTRACTOR	NEWCCCCUYC	TCCTACTTAG	ACGCGGGCTAC	TGACCTGCGC	TOGGCCCCAA	GCCGACTCGC	
7001	COCACOTTIC	CCGGGACCGA	CCCITACCCCT	CRECKRETATE	TUTCOGCTC	ACCIATICANAC	TOCOCCCIECT	ACTEGACOCO	ACCCCCCCCTT	COSCHOCOC:	
11701	CUTTAGAGACA	OCTOBOCCO	GACCTOROCT	GACCAGTOGCA	ניברינינינינינינינינינינינינינינינינינינ	CTGGCAACGT	CONCURRENCE	GACCAATATO	ACGROGACOA	TOACTACGAG	
	COACCTOON	- משכנכנמפנ		CCGCCACCGT	COCICUCIOCIC	GACCGTTGCA	OCCOCCOCAC	CTCCTTATAC	TOCHOCTOCT	ACTCATOCTC	
11801	CCAGAGGACG	GCGAGTACTA	AGCCCATOATO	TITCTGATEA	GATICATICICAA	GALFICAACIDG	ACCURREDGE	0000000000	CTGCAGAGCC	AGCCGTCCCP !	
	OOTCICCTOC				CTACTACGIT	CHACITHECE	TREGREETHICA	כמכככמכנמכ	GACCICTOGG	TCGGCAGGC	
11901	CCTTAACTCC	: ACCOUNCEACT	accechiant	CATOGACCIAC	ATTATETER		CAATCCTMAC	OCUPILICADO	AGCAGCCGCA	CCCMCCD	
1	CONTTORO	TOCCTOCTOA	CCCCCCTCCA	GTACCTGGCG	TACTACACK	ACTOACGCGC	GTTAGGM:TO	COCANGOCCO	restences	CCGOLLIGGG	
1000		STATE STATE OF THE	appropriate the constant of th	CHITACORCIACAA	ACCCCACCCA	CGAGAGGTG	CTCCCGATY:G	TANACOCCCT	GGCCGAAAAC	AGGCCATCT	
TOOPT		_			TOCOCTOCOT		GACCCCTAGC	ATTTOCOCCA	CCCCCTTTG	TCCCOOTAR	
10101	and the same				מכמכנו 160כד	CCTTACAACA	GCGCCAACGT	GCNGACCAAC	CTUBACCOOC	TOTOGGGGA	
10177	Macre Control			GCOACGAMOT	COCCCACCGA	CCANTISTEST	COCCUPTOCA	concroored	GACCTROCCCG	ACCACCCCC"	
,000			Acres March		CAGGGCAACC	TOCOCTUCAT	GGTTGCACTA	AACOCCTTCC	TOAGTACACA	OCCUCCAN.	
10221	TOTALCACOA	· rastardada	TYCKACTYCE	-	grecentos	ACCCUAGGTA	CCAACGTGAT	TTCCOGNOO	ACTICATION	COCCIONATION	
•	ALACOCOCIO	Sacratage .				AATGGGGGT	מאמעכעכנפכ	AAAGTGAGGT	OTACCAGNET	GOTICE AGACT	
12301	OTOCCOCO	GACAGGAGGA		AAAFACTCGC		TTACCACTGA	CICIOTOCO	TTTCACTCCA	CATOGTCAGA	CCCCCTCTGA	
	CACACACAC			-							
						CHATTLANA	ACTROCAGES	CCTCTCCCCC	CHICCOCCTC	CCACAGGCGA	
13401	ATTITION	GALCAGIAGA		CAMARICATIC MUNICIONAL	GGACTCATTC		TOMOGRACE	CCIACACCCCC	CACOCCCGAG	OCHETECRICT	
		· manager					CHECKTICACE	CACACTOCCA	aconstaces	CGACACATAC	
10691		CACAGATCGA				ACGATTATCG	CYTHANACTURE	CTCTCACCGT	CCCACAGGGC	CCTOTOTATG	
	200					CHALLIAGEAT	NCTITICCACO	AGATTACAAD	TOTCAGCCOC	COCCULACION	
13601	CTAGGICACT	T TOCHORCACT				CCTVX:TCGTA	TEMMOCTCC	TCTAATGTTC	ACAGTCOGCG	COCOVCCCO	
	CATICAL LA							•	Proged		
		· variation .	CHAST BACT	TAAACTACCT	CHILLIANTANG	CITACITACAGA	AGATOCOCTO			ACCACICAGO	
10/21	MOUNTAINCAL TO THE THE THE					CUCANCETOR	TUTAKKATAG	CAACGTGTCA		PCCPCCP(19)	
	1000000				ATTACKET	THE STANTING	CARCITICATO	CTURRACATOR		CATCRACTA	
12801	GATTITOCO	D ATGCACGTCG						GACKTIGTACT	GCCCCCCTT	GTACCTTOOL	

PHRKAd5gag MERGR2

12901	OCCATOTATO	CCTCAAACCA	OCCUPANT.	AACCASCTAA	TYSACTACTY	CANTONIA	CONTRACTOR	ACCOUNTABLE	THEACCAAT	Carrayment
	CCOTACATAC							TYSTOCKETCAT		
13001			: cetentrici	. אכאכנגאאאו	ATTRICIACACITE	אדירניאניונ: ניייהארידה	ACGATGRATT	CENTERODGAC	GACATAGACG	ACAMIGRETT
	TOCOCOTOAC	: CGATGOCOGO	GOACCAAAGA	ו אאנוטכנינכ	エカカのに・かいこう	CXXXITTECEAT	TGCTACCTAA	GGAGACCCTO	CTOTATCTGC	TOTOCCACAA
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13101		COCCAGACCC	TOCTACATT	. מכשעכשיבוני		GARCAGREAG ARXXXXXXXXXX		GOGDANGGAN AGCTTCCCCCA	GGCCNAGCAG	CPTGTCCGAT
	AGGGGCOFF	* OCCOPATIONS	ACCUATOTICAA	בנידוטוכנכנ	C. Delicate Control	Trunchichina		CUCTITICCTT TCGMOGCGT		
13201		COCCCCCCC	OTCANATICET	AGTAGCCCAT	TTCCANGGTT	GATAGESTET	CTTACCARCA	CTCCCACCAC	CCCCCCCCCC	CTCCTOTICO:
	DATICCOCCOAC	: מככממממכמכ	CAGTCTACGA	TCATCOCCTA	AAGCTTCC:AA	CTATCCCAGA	GANTOCICCT	GAOCOTOGTO		GACCACCC
			Pst							
13301	ACCACCACTA	I CETAMERAC		לבטביוהני מאכההכתמהה	CGANANANC	CHICCTCHIC	CATTROCCA	CAACGOGATA	GAGAGCCTAG	TOCACANIA1
	TCCTCCTCAT	· COATTIONS	AGCGACGACG	TOGGOGICOC	CCTITITIES	מעכשטענונככ	GTAMORETT	GITGCCCTAF	CICICOGAIC	ACCTI-ITTCTA
13401	OAGTADATOG	A ANGACOTACO	COCADGAGGA	CAMOGRACITA	CCAMICCTIGG	GUCCIACCAC	COSTECUTEAN	AGGCACGACC	GTCARCOORD	PCTOGRESION
	CTCATCTACC	: TICTOCATOC	degreerent	GICCCTOCAC	פטוגולנולאספ	טנאנטטאנפונט	GOCAGCACTT	TECGTOCTOO	CAGTCOCCC	AGACCACACC
13501	CAGGACGATG	ACTCOOCAGA	COACAGEAGE	OPCCTOGATT	TREGRENOATING	TAXCAACCCG	TPROCACC	TTCGCCCCAG	CCTCGCCACA	ATCTITANA
	CTCCTGCTAC	TOAGCCUTCT	OCTURED OF	CAGGIACCTAA	ACCEPECATE	ACCGFTCGCC	AAACYACGTC 10	AACCOGGGTC	COACCCCTCT	TACAMAITT
13601	ANANAANAAA	GCATGATGCA	ANATANNAA	CTCACCAACA	CCATTACACC	GAGCHTTRIGET	TENCHASTAT	TCCCCTTART	ATCCCCCCCC	COCCIONTETA
	Thursday.	COPACTACGT	TTTATTITE	CAGTRICTICC	GGTACCCOTTOG	CINCOLACCA	ANAGAACATA	AGGGGAATCA	TACGCCGCGC	OCCULTACAT
13701	TOAGGAAGGT	· cercerecer	CCFACCIACIO	TOTAGACACC	הבושמונוניה	TEXTOGUAGE	CXTRAGGENCE	CCCTTCGATO	CTCCCCTOOA	CCCCCCTTT
	ACTCCTTCCA	GOAGGAGGGA	GGATGCTCTC	ACACCACTCG	COCCOCOGIC	ACCCCCCCCG	CGACCCAAGA	CCGAACCTAC	GAGGGGGCCT	GOCCCCAA.
		Kirel								
13801	oraccrecoc	GGTACCTGC	OCCTACCORD	GRIGAGAAACA	CEATCORTA	CTCTGAGTTG	GCACCCCTAT	TCGACACCAC	CCGTOTGTAC	CTGGTGGACA
	CACCCAOCCO	CCATGGACGC	COGNICCCC	CCCTCTFIGE	CCITAGGCANT	CARIACTERAC	CCTCCCGATA	ACCIONAGIO	COCACACATO	GACCACCTOT
13901	ACAMOTICAAC	_		ACCAGAAAGGA	CCACAGGAAC	TTICTGACCA	COUPLATICA	AACAATGAC	TACAGCCCGG	GRGARGCAAG
	TOTTCAOTIC	CCTACACCGT	NOCCALITINA	TREFFERE	AGTOTOOTTO	AMAGACTERT	CCCAGTAAGT	TTYCHACTO	ATCTCKGCC	CCCTCCCTT.
14001	CACACACACC	ATCANTCTIO	ACGACCOATC	GCACTCACARC	CKICHACCTRIA	AAACCATCCT	CCATACCAAC	ATCCCARATO	TOAACGAGTT	CANTITION
	Grotorcroo	TACTTACAAC	TGCTCGCCAG	משפעכבככם	CCUCTROACT	TTTCGTACTIA	COTATGGTTG	TACOCTITIAC	ACTITOCICAA	GTACAAATTA
14101	ANTARCITIA	AGGCCCCCCC	GATCCTCTCG	COCTROCCTA	CTAAGGACAA	TYACIFICACI	CTCANATACG	AGTGGGTGNA	OFFICACOCTO	CCCCANAXACA
	TTATTCAAT	TECGEGECEA	CTACCACAGC	OCCUNICACYT	GATTCCTVITT	AGTECALITIC	GACTITIATIC	TCACCCACCT	CAAGTGCGAC	GOCCTCCCOT
	-			•	Pvad					
14201	ACTACTCCOA	GACCATOACC	ATMARCCTTA	TRANCANCRC	CATCHINGAG	CACTACTION	AACTGROCAG	ACAGNACOCO	GTICTOGAAA	CONCATOO
	TOATGAGGET	CTOOTACTOO	TATCTICAAT	ACTAGENCO	CTACK:ACC:TC	CHEATGAACT	THEACCOCHE	TOTOTAGE	CANGACCTTT	CIRCHITAGEC
14301	OCTANOTITE	GACACCCCCA	ACTTCAGACT	CONTITIONS	ددسسدسي	CTR.TTR.TR.AT	CCCTTAXXXTA	TATACAAACO	MOCCITICGA	TCC NGACATU
	CCATTICAAA	CTOTOGOCGT	TOMOTOTON	נת:יועער אסטט	האאראהאיאה הייההייאאא	ניאניאיניאינדא	CHANCECAT	ATARCTERIC	TTCOCIANGET	MACTETERA
14401	ATTITIOCTUC	CACCOATGCGG	CONTRACTIC	ACCCACACKC	מובליימוא:אא	CTTCTTCTCCC	ATCITICANCC (CATABOCCITY	CCAGGAGGGC	TTTAGGATCA
	TAMARCGACG	STECTACOCC	CCACCTRAAR	TOXATOTOG	COCACTOCAT	מאכאאכבנג	TAGGCCTTCC	CCUTTCCCAA	BOTCUTCOG	AMTERTAGE

Figure 15I

CCCCACTORY TOCCATTORY: ACGOTANGED GAGARICCT CTCTTCGRA	GEAGI-TG-TI-A COTCHACCAT P CTACTGGTCH GATGACCAHC	OTGRGGTTCT And And TTTTGGGGYY	AGTCCAGCIA TCAGGTCÓCT	AAAACTOTTT ACCAACACCO TGGTTGTGG91	CCACCACCI ANATOANCA TITTACTICT GCACCTRRICT	CGCGCCATT CGCCCGTAAA GCGCCGATAAA GCGTTAATCT AAGAGATACT TTCTCTACGA
				-		
CHACAGGGCG CHACACCACA TRANCOATCA ACTTGCTNGT ACCCGAGGTCAGT	ACCCAGINCC TOGGICATOS COGNOCASOF GCCTOGICCA		OCATCOGAGG CGTAGCCTCC	CTCGGCGTCG AAGCGCTCCG TTCGCGAGGC	CCATCANCYC GGTAGCTATGCT CGCGATACGA CGCGATACGA	CCCCAGCAGCAGC CCCCAGCAGC GCCCCCCCGG GCCCGCGCGGGGGGC AAAATCAAAGTTTC
AGATGACAGG TCTACTGTGG GTGGAGGACA CACCTGCTGT CCCCTGGGGA					GTCGNTGACG CAGCTACTGC GCCGAGCCCG CGCCTCGGGC	
CONGCTTOM GCTCGAACTT AATTSTAKGCG TTAGGTTYAG GCTTACGTTG	-				GCCGTCGTCG CCCGTCGTTC ACCGTGGTTC TCTCACGACGC	
GTTMCCMH GSZMTSTTC FMKTFRKE GTCHITFEG MATERITAL	TACANCCTAA ATUTTCSATT TACTTTCCAC ACTAAACGTG				GCCGCTCTCTCCCCCCCCTCTCCCCCCCCCCTCTCTCCCCCC	
CRIANTINGAC CCTACATO TICAACGITA AGGITIGGES AGGITIGGAACGI TCCGACTITE	MARCHAGAT CTITOCHICA TCCTCTCTT CTITOCHICA CCANATCCC TCATGGATCC GCCTTAGACA AGTATCTTAGA				CACTATOCOC CACTOCACOC CACTOCACOC	
CCCCACACTICATO GGCGTRANCAN GRANINGRANC CCTTCTTG ANGREGICATO TTCGCGGGAC			-	TGCCCCTACG ACCACAGATGC ACACAGAGGTG TGTGTCCGAC	CTANGGOCGCG GACCTCGCGC CCAGTGTTCA GGTCACAGGT	
CCATTACATTC CCATTGTANG GCARTGTCCCC COTTGCCCCCC GGCTGACGAG CCGACTCCTC CCGACTCCTC	CCCCTTACAG CCCCTCACAC ACCCTCACAC TGCOATCTG			ACGCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC		GCNGCOCTOG GCNGCOOTOG CNGNGGANG GNCTCCGA GNCTCCGTTO GNCTCGTTO GNCTCGTTO
TCTGGAGGGT AGACCTCCCA AACACAGTO TTGCCACAC AACGGTGTGC	CCACTAGTTT CCACTAGTTT AACTACGCC TTGATGCCCC	TOATGCALGA ACTACGTTCT COACCAGGCC	GCTGGTCCGG CCCCACCATCA GCGTGGTAGT	CTGACGCCAG GACTGCGGTC CCTTATATCG GGAATATAGC	CCCCOOCACT GCCCCCTGA ACTACACCCC TCATGTOCCO	SHI ATTOORDECTOR CTCROODICG CTCROODICG CANTOCCOOL
CCTACGATCA GGNTGTACT AGGCGCCGC TCCGCCGTCG GGCGACACCT CCGCTGTGGA	ADAMDAACC TCTTCTTTOG Kpm CCTTGCATAC GGAAICOTATO	TTGCCAGACA AACGGTCTGT GCTTCTACAA	CCCCCCAGCC	CACTOUTANT CACTOUTANT CCATOTCCAT	AOTOCOCOTO TCACOCOCOC GAGCOCOCO CTCCOCOCOT	GACCACCAGA CTGCCCCCCC TGCCCCCCCG AGTGCTATGA TTGCAAGAAA
14501	14801	15001	15201	15301	15501	15701 15801 15901 16001

Figure 15J

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16101	CCAGGTCATC				מאתאטנאטנ	ATTACAAGE	CTGAAAGCTA	ANGCOOGICA	ANAGGNAAN	DAMAGATRIAT
	COTCCACTAG	מסכסככרוכד	AGATACCCOC	ASSCHIPCTIC	בידוז יועימיזיכ	דאאזיידיניאן יאיכדידיניאדר	CCCTTTCCAT	THEOCECAGE	THEFT	CTTTCTACT.
								Sall		
16201	GATGATGAAC	TTGACGACGA	CONTRAMETE	CTRCACCCTA	ניג בניטבור ביני אונ	パエロいしいいいいい	CACTOGAMA	GTYCOACCCOT	ANACOTOTT	PTGCGACCC"
	CTACTACTTO	AACTGCTGCT	CCACCITICAC	GACGTOCGAT	נאשנטנונטנונונו	FXCTYCC('AT	GICACCTITIC	CAGCTGCCCA	TTTTOCACAA	AMCCCTOCHO!
16301	GCACCACCOT	AGTETTTACO	CCCCCTTCAGC	CCTCCACCCG	CACCITACAAG	CACATATATA	ATGAGGTGTA	CHOCCACTIAN	CACCTOCTTO	ACCAGGCCAA
	COTOOTOOCA	TCAGANATGC	PROCEAUTOR	CGARGTTAGG	CHUCATGITC	CCTCACATAC	TACTCCACAT	OCCUCION	CTCGACGAAC	TCGTCCGGTT
16401	COARCOCCTC	COCCACTETO	CCTACGGAAA	CECCICATANG	CACATICITIES	いないないない	CCACCACKKC	AACCCAACAC	CTAGCCTAAA	OCCCGTANCA
	OCTCOCOGAO	CCCCTCAAAC	COATCCCTTT	COCCOTATIC	CTGTACGACC	GCAACOGCTA	cercerces	TTCCCTTCTC	GATCOGATIT	COCOCATTEST
	P#4									Knnt
16501	CTUCAGCAGG	TOCTGCCCOC	OCTIONACCO	TCCGAACAAA	AGCGCCGCCT	ANACATACCING	TCTOSTISACT	TOOCACCCAC	COTOCASCTO	ATTENTACECA
	DACOTCOTCC	ACCACCCCCC	CCDAACCTCGC	AGOCTACTT	TCGCGCCG3A	TFRUITCH	AGACCACTOA	ACCGTOGOTO	GCACOTCOAC	TACCATURGE
16601	ACCCCACC	ACTOGRAGAT	GTCTTGGAAA	AAATOACCCT	COAACCTORS	CHEROMICCEG	AGGTCCCCOF	GCGGCCAATC	ANGCAGGTGG	COCCGODACT
	Tracocarcac	TOACCTICTA	CAGAACCTTT	TTTACTORCA	CCTTRIBACIC	ONCCTCOOCC	TCCAGGCGCA	CCCCGGTTAG	TTCGTCCACC	GCCCCCTGA
16701	OCCUTOCAG	ACCOTODACO	TTCAGATACC	CACTACCACT	AGCACCANTA	THYCCACCOC	CACAGAGGGC	ATOGAGACAC	AMCGICCCC	OGHISCUTCA
	CCCOCACOTC	TOCCACCTOC	AGTETATES	CTCATORITCA	TCGTGGTCAT	AACGGTOGCO	OTOTICACCO	TACCTCTOTO	THOCAGOO	CCAACGGAGT
16801	gcaaraacaa	ATOCCOCOOF	OCACCCCCCTC	CCTGCCCG	CGTCCNMAC	CTCTACCAAG	GITICAAACGG	ACCCUTODAT	GTTICGCGTT	TCAGCCCCC
	COCCACCOCC	TACOOCUCCA	COTCCCCCAG	CCACCCCCCC	GCAGGTTTCTG	GAGATACCTC	CACOTTINACE	TOGGCACCTA	CANAGCOCAA	ACTCOCOCIC
16901	000000000	CCOPTCOAGO	ADIGTACTOCCO	CCCCCAGCGC	GCTACTGCCC	DANTATRICCC	TACATCCTTC	CATTCCGCCT	Accessor	ATCGTGGCT
	CCOCOOCOC	OCCAROCTCC	TTCATGCCOC	OCCOOLCOCO	CCANTINCOO	CTTATACGGG	ATCTAGGAAG	CTAACGCCGA	TOCOCCCOA	TAGCACCGAT
11001	CACCTACCOC	CCCAGAAGAC	GAGCANCTAC	CCCACCCCCA	ACCACCACTO	CAACCCCCCC	ככנאיניגשכפכ	CCTCCCCAGC	CCGTOCTAGE	CCCGATTTCC
	GTGGATGGCG	OCCUPACIO	CTCGTTCATO	GOCTGCGGCT	TOGTOCTOAC	CTTORGEGGG	COCCCCCACACC	OCAGCOGICG	COCACGACCO	GCCTANAGC
17101	GRECOCAGO	TOOCTCOCGA	ACHIAGCECAGG	ACCCITAGING	TRECARCATE	NYCHACCAC	CCCAGGATATCG	TITAAAAGCC	CONCINION	OTTCH TOCAG
	CACOCOTCCC	ACCOAGCCCT	recreecence	TCACCACCACC	ACCEPTIGICS	COCCATGGTO	GGGTCGTAGC	AAATTTTCCC	CCAGAAACAC	CAAGNALGTY
										Page.
17201	ATATOOCCCT	CACCTOCCOC	CHECGRIFICE	COCTRCCCOC	ATTICKGAGGA	AGANTIGCACIC GTAGAAGGGG CATGGCCGGC	GTAGGAGGG	CATGGCCGGC	CACOCCCTOA	COCCURRCAT
•	TATACCOCCA		GAGGCANAGG		TAMORICTECT	TCTTACCITCO	CATCCTCCC	GTACCOGCCG	OTGCCGGACT	OCCCCCCCTA
	1				Sphi					
17301	O TOTAL CONTRACTOR	CACCACCAGC	COCCOCCCC	GTCGCACCOT	CCN. ATHRCINCG	GCCCTATCCT	DECEMENT	ATTCCACTOR	TCCCCCCCCCC	מאדדססכמכ
	COCAOCACC			CAGCGTGGCA	OCCITACOCYC	CGCCATAGGA	CCCCCCACCA	TAAGGTGACT	AGCGGCGCCG	CTAACCCCCI
17401	GHACCCAGA		GGCCTTGCAG	GCCCAGAGAC	NCTICATTINA	AACAAGTTYC	ATCTINGARA	ATCAMATA	ANACTECTOCA	CTCTCACGET
•	Checonary		CCCCANCETC		TOACTAATT	THEFTEANCE	TACACCTITIT	TAGITITATI	TTTCAGACCT	GACALITOCCIA
										Ecofiv
17501	CALLEGER	TOTANCTATE	TTGTAGAATG	GANGACATCA ACTITICOGIC	ACTIFICATION	PRITIGATETED CICACACTOCT CACOCECUTT CATOODAAAC	המאכאממסכד (CACACACATE		TRECONGRATA
	OCCIMICA 60		AACATCTTAC	CTTCTGTMGT	TICHANGGENG NGACCGTACAC	AGACCIARGAC	OCTOTOCCCGA (GCGCCCCCAA		ACCOPTICTAT

Figure 15K

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	Ecoffy									The Best Page 27.
7601	PCCCCACCAG		GATAGCGCCT					THECACCINT	TELEBOTEC	CCTCGTTCC
	AGCCOTOOTC	CITATACTCO	CCACCGCCCA	MUTHICACTICE	פאההואותיאי			W. W. W. W. W. W. W. W. W. W. W. W. W. W		
7701	CTOGAACAGC	MOCACAGAGC	AGATISCITIANS	CONTABOTTO	AAAGAGGAAA	ATTRICARCA		GATGGCCTGG		TAGCGGGGF
	GACCTTOTCO	_			THE THE STATE OF THE	TAMAGGTTGT	THICCACCAT	CTACCOGACC	CCAGACCGTA	ATCGCCCCM.
					(firefill		•		1	
7801	GTYACKTEC	CCAACCAGGC	ACTCCNUNT	ANGATTANGA	GTANATHGA	חברככנכנכר" ו	CCCTTAGAGG	AGCCTCCACC	COCCETOCAG	ACAGEGECE
	CACCTGGACC		TCACCITITITA	TTCTAATTGT	CATTUTANGE	ACESTICITIESA	CHANCATCTIC	reconsortoo	CCGGCACCTC	TOTCACAGY
1901	CAGAGGGGCG		CONTCACACIC	CCGACARAGA	AGAMCTOTA	THINGS WAY	TAISACGAGGC	recentoring	CAGGAGGCAC	TANACE ANT :
	GICTICCCCOC		-	GRETETECET	TCTTTTAGAG	CACTORCETT	ATCTGCTCGG	AGGGAGCATG	crecrecond	ATTICETIC
1000				CCTANTOGA	מאין וייסיבכ	NATACACC	COTAACCCTC	GACCTGCCTC	CCCCCCCCOA	CACTICAGEA":
7000	COACGOOTO		-	AGCOCOGOTA . CCGATYXCCT	CACCIACCOCK		GCATTGCGAC	CTOGACOGAG	0000000000	CTV:CCTCCTV:
		Mary Mary	Character Control	GTTGTAACCC	GICCTAGCCG	COCCIPCCTO	בפככפכפטבפ	CCAGCIZOTCC	OCCUNICATIO	COCCOOTAG
10101	THOCACACO		-	CAACATTORIG	CACCIATICIATIC	RECEPORANC	מכסטנמנמסכ		COCTAGCAAC	GCCCROOCATC
18201	CCANTOOCAA	_	-	CCATCCTCCC	rereseasts		AGCOCCOALG		TAGETAACOT	Greenwagen
	CONCACCON	_	TOTOACTION	COTAGCACCC	MUNICOCCON		TECEGGETIFE	TACCAMGACT	ATCONTICCA	CARAINE
8301	TOTCATOTAT	GCOTCCATOT	CCCCCCAGA	מנאחכיוסכיום	ACCICCCION	כנובכנינוכיווו	CCAMGATAGC	TACCCCTTCO	ATCATGCCGC	MOTOR POLICY IN
	ACACTACATA	CGCAGGTACA	GCGGCGGTCT	CCTCGACGAC	TCCACCACC	מנטטטנטאוו	CASTICTACEG	ATGGGGGGGC	The Income	
8401	CATGCACATC	TCGGGCCAGG	ACCCTCGGA	GTACCTCACC	CCCCAGCTGG		כבמכמככעוב	GAGACOTACT	TCAGCCTCAG	ATTICITIES
	GTACOTOTAG	ACCCOUNCE	TOCOGAGCCT	CATCHACTCG	CHECCEGACE		Secretary Secret	CILICARA		***************************************
18501	MGAAACCCCA	COSTOSCOCC	•	GTGACCACAG	ACCORPACTOR		Chacamete	*CCCTOTGGA	CCCTCACCAC	TEACGCATGA
	TCTFTGGGGT	GCCACCGCGG	ATGCOTGCTO	CACTOSCHOTIC	TOGCCAGGGT		CACCCCANGE	AGGACACC	OUT THE STATE OF T	STATE OF THE PERSON OF THE PER
18601	COTACAAGGC	OCCUPATICACE			TOTOCTOGAC	ATGGCTTCCA	CGTACTTICA	CATCCGCGGC		CCCCCCAN
	OCATOTICCO	COCCANOTOG	GATCGACACC	CACTATTCOC	ACACGACCTG	TACCGANGGE	GCATGAGACT	CIACUCCO		TCBARTORS
18701	TITTAAGCCC	-		_	CCCANGRATIG	CCCCAAATCC	PROCOANTO	CTACTORGETG	CTACTOCICY	ACTITION ACTION
	AAAATTCGGG	-			ממפדאכותיאונ	CARCITIMA	ACTION THE PARTY	THEOCAGO		COTATABATA
18801	CTAGARGARG			CAAGTACACG	WACANIAL TON	COLCOTOTT	TriActricA1'A		_	CCATATTTAT
	GATCTICITIC	_	-		Porter Param	CONTRATABLE	CATTICARCE		ATACOAGNAT	CTCAUTGGTA
18901	TTACAAAGGA			AACGICAAAC	TEGNITIVIA	CONTINUE	GTANGETICS		TATECTETTA	GACTTCACCAT
	AATGHTCCT	_				CAATGAAAGC	ATI:TTACOGT	TCATATOCAA	AACCCACAAA	TOAMANTON'
10061	COAAACAGAA	ATTAATCATO	CAGCTGGGAG	TCAGOATTIT	TICTIONTOG	CETTACTETOS	TACAATGCCA		THOOORTH	ACTITITACE"
	GCTT1G1C11			_		CHINANTCEAN	TITTICICAL		-	AATCOTOAT .
19101	CCCOPTCCOF	NAGAACATIT			-	CLTTTACCTT	NAMAGATT			TTACCACTA!
19201	ACTTGACTOC	-	TIGINCAGIO				ATATTACTTA	CATGCCCACT	TAATTCCTTC	CATTGAGTO:
) } •	TCAACTCACC	ATTICACCAT	- ANCATOTICAC	TICTACATCT	ATATETTEG	CASTUTACTIONS	TATAMAGAME			

Figure 15L

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												_	. O.	_	
GGGTAATATAC CCCATTATAC TCCATTGGGTS AGGTAACCAC	AACTICCAAA TTGAA'#:T'IT'	TGCTACAGAA	AACATAGECES TTGTAREGES TAGETECEES	ACCGAGGGC	GGACGCGATH GCGGCTTCAT	GGCCCGAGTA	ACANCTA'	GAANTHOC!	OCOCCIALITY:	TACCTTCS I/ A	CAMCTITAA	TANCCGATCA	CCCITACTANIC	COCINCOATTO	TCCAGTAAGT ACCTCATTGA
		-												900	
ACAACAGCAC TOTTOTCGTO TITTOCTTGAT AAACGAACTA	ACTGAAGATG TGACTTKTAC	CCCTITITICT	CCTGTACTCC CCACATCACC	TTCOCTCACC OCAATGCTOS	COTTACGACC	GUANGAGGAC	OCCAGE ATTA	ACCACCAGTC TGCTGGTTCAG	CCCCCAAAG	GGGATGGATC	COCOCTTOCT	ATTGATATTG	CACCTACTAT	CTOTCCOORT	CARCCATTC
CTANTGRATT GATTACATAA CATACCAGGT GTATGOTCGA	AAATCATGGA TTTAGTACCT	CHITTACCTA	CCTCTTTAAA	GATOTACTTO	1100100100	AATTTTTGGA	CCAACTUCCT	TTGCTGTGGT	GOCCOTTGAC	ACCGAGATAT	CCCCACCAAT	TTTACGATCG	GOCAOTICCAC	TACACACTIC	CCCTTOGICO CATCCCATTC TCCMITAMIT GIGANACCOC GTACOCTANG AGITCATTOA
TTTTATTOTT ANANTANGGA ACAGANGCTTT TGTCTCGAAA	GAATTATTGA CTTAATAACT	AACAGGTCAG TTGTCCAGTC	COGITICACA	TOTOGRATOCT		ANGANACOGE			-			-	TCGOOTACTC		TTACCCADA ANVITTETT TECHTOCA ANTORITETT TITEMMOA ACCETACOOF
TTAGRENIA ANTERETITE ARAGAMANE TERRITETE	CCACATEITA	TAMMACCTAA		CTATIOGUTT		COCAGICTIC	TCCCTAGGAA					-	TCTTTCMCG		GTTGACAGCA TTACCCAGAA AAAATTTCTF CAACTGTCGT AATGGTTCTT TTTCAAAGAA
TACATHGCTT ATGTAACGAA TAGATTTOCA ATCTAAAGTT	CAGCTATGAT GTCGATACTA	CTTACCAAAA GAATAATICC	CCATCATAAAT	TTTTTAMGA		ACMITICARTI TRIMACITCE Pall	- <					-	GAGGAAGAAA		TTACCCAGAA
CAGGICCTAAT TACATHGITT GTCCVEATTA ATGEAALGIAA AATGEGTTG TAGATTTOCA	ACCUPATION TOCK	TACAGAGACT	ANTANTETIC TTATTAVAC	CTTCCAACGT	TOTGACCAGG	CACCCCTTCC	TTAACATGGT AATTGTACCA							ACACAACAAC TOTOTTOTTO	CAACTCTCGT
CTATCICCICAA OATACOSCITT ATCC:ACTTC	ATTITICIDANTC TACACCTTAG	GTGTGATTAN CACACTANTT	AAGAGTTGAA	AAGTACAGTC	TOGANCETES	TGGTCGCTAT ACCAGCGATA	ACCINCTAC TECTTECTAC	PACCECACET "TETTECCEAT ATCCOSTODA AGAAGGGGTA	GCTCTACCCT CGAGATGGGA	ANGRAANCEC FICCTFFIGG	AGANOCITOCO TETTECACCO	CCCANTRITIO		ACCTACACCA AGGATGTGGT	CAAGACCTATA
CCCCAACAAT CCCCTTCTTA CCCCCTACTTA	GENCETITICE	CCACTGRAAG	AAANTGAAAT TTTTACTTTA	CCACAAGCTA	TOCTACATTA ACGATOTAAT	TOCTOOGCAA	CACCTTOANG	TACCCCACCT	CCCCCACAT	CCTTANGACT	CACACCTITA	TTGACCOGGA AACTGCCCCT	TATECCAGAG ATAGGGTCTC	CAGGIGGGCA	CCCTTATAGG
AGAACTAANTO TCTTGATTAC CCHGTTCTGG			TTTTCAGATA AAAAGTCTAT	TOTATTTGCC ACATAAACGG	OCTADTOGAC COATCACCTO	COCTCAATOT	ACACCTACGA TOTOGATOCT	CATTROCCTT	TATCTCTCCG ATAGNOAGCC	CCTTCACGCG	TTACCTCAAC AATGGAGTTG	AACCCCTCAC TTCCCCAACTC	AGGGCTTCTA TCCCGAAGAT	CCTCATOGIT	TTCCCCTATC AAGGGGATAG
19301	19501	19601	19701	19801	19901	20001	20101	20201	20301	20401	20501	20601	20701	20801	20901

Figure ISM

Figure 15N

PNRKAUSgay MER682

=	TRETABACTG ACGATETGAG		CICCATANC COTTE	ונכאא	NGCGA NGCGA	CCACTCTACA			ATTENTOARA ANDOTTOCKET TAAATAOTAT TACGARGKEN 1940 CAAATGATTO CAAATGATTO	ATGCTTCCGT TACGARGGCA Fell CAAACGACTG
AAGCTCGCCT TTCCGAGCGGA Psfl	AAGCTCGCCT TTCCGAGCGGA Psfl			CCCTCCCCAC	CHEXXING THE	Certification			CACTOCACAC	OTTRICTUM.
CAGGIACCEC TOCAGGIANTE GEOTICATEAT GIECLATGEOD ACOTECTING CGROTAGIA	TOCAGGAATC			OCCICCATCAT COTCACAAAG COXXOTAGTA GCAGTGTTC	CAGAACAACG	TCSTTCSACTTCCSA		GCCCCACGA	GGAGCAAGTC	GTILCAGAAC
CATACOGCO CCAGARCTPC CACTICATED O GTATGCCAGT C GTCTCGAAG GTGAACCAGT C FALL MANNAMA	CCAGAGCTTC GGTCTCGAAG	CACTICATA O GIGAACCAOT C	00 (CCCACATAGET	TRANGITICIC CITTAGATCO ACTICAAGCO GAANTIAGÓ	CTTTAGATCG GAAATCTAGC	TTATCACGT	CCATGAACAG	GTAGTCGCGC	COCOCOTCO 1
CCATGCCCTT CTCCCACGCA GACAGGATGG GGACACTCAG CGAGATTCATT: OCTACGGGAA GAGGGTGCGT CTGTGCTAGC CGTGTGAGTC GCCCAAGTAG	CTCCCACGCA		CO	GCACACTCAG	CGGGTTCATK: GCCCAAGTAG	ACCOTANTET TXCCATTAAA	CACTITICCEC	THCCC-TOOK AGCCACCCG	ACARCCACATA	CCTCTTGCOT
CCOCATACCA COCCOCTO COTCOTCTTC A CCCCTATGGT CCCCTATGGT CCCCTATGGT CCCCTATGAC T	COCCOCTO CONCOTOTO COCCOCTO	CCAGCAGAAG		ATTCACACACOC TAAGTCGGCG	rgcactratoc GCGTSACACG	GCTTACCTCC	TTTRCCATRC AAACGGTAGG	TTCATTACCA AACTAATCGT	CCUGICOGUIT	CONCITTION IN
ACCATTIGIA GCGCCACATC TICICITICIT TO TGGTAAACAT CGCGGTGTAG AGAGAAAGA AG	CCCCCACATE TACTETATET CCCCCCACATE AND AND AND AND AND AND AND AND AND AND	THETETITET	-	PCCPCKKTOT AGGAGTGACA	CCACCATTAC	CTCTCTCTGAT	GRECTEGECUET	COCCOMICC	ACAMOGOCOC	ANGAANNGA
TETTOGGEGE ANTOGGEAAA TECGGEGGEG AA AGAACCCGCG TTACCGGTTT AGGCGGCGCT TE	ANTOGCCAAA TCCGCCGCCG	TCCGCCGCCG AGGCGGCGCCCC		ACCIOCTACC TCCAGCTACC	CCACCACACTO	CCACACCCCC	GCACCAGCGC	CACAACACTA	CTCAGAAGAA	CCACACA ACCT
CICCATACGC COCCTCATCC OCTITITIOS GO CACATATATOS CO	COCCICATCE OCTITITATED COCCANOTACE	CONTINUES	88	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	CONCOCCACC	CCCTCCCCCT	CERCECTECTO	ACGTECTCCA TCCACGAGGT	ACCAACCCC	POCAGCOCK TOCAGCOCK
COCOCTCOGO COTCOTTCO	COCOCTCOGO COTCOTTCO GCOCGAGCC CCACCANGC	CCACCANGC		COCTOCTCCT	CTTCCCGACT	GRECOTTICE	TTCTCT TATA	CCOTCTTTT	CHAGTACCTC	ACTOR ACTOR
CCTAACCGCC CCCTCTGAGT	CCTAACCGCC CCCTCTGAGT	CCCTCTCAGT		TCGCCACCAC ACCGCTCGTG	CONTINCACC	GATGCCGCCA	ACCCCCCTAC TOCCCCCATO	CACCTTCCCC	CAGCTCCCTO	CCCCGCTTGA
CACTANTAGE TEGIECTOOG	CACTANTAGE TEGIECTEDE	AGCAGCACCC	2 F	NOCTETEGTA TCCAAAACAT	AGCGAAGACG TCGCTTCTGC	ACGAGGACCO TCCTCCTGGC	CTCAGTACCA GAGTCATGGT	ACAGAGGATA TOTETECTAT	AAAAGCAAGA TTTTTCGTTCT	CCAGGACAAY COTCCTGTTS
OCACAGGGAA ACGAGGAACA AGTCGGGCGG G	ACCAGGAACA AGTCGCGCCGG	AGTECCOCO		CCCCTGCTT	CCCTACCGCT	CTACCTAGAT	GTOOGAGACO CACCCTCTOC	ACGTGCTGTT TRCACGACAA	GANGCATCHS CAGGGCCAUT	CAGCGCCAUT
CTOCOACGE TTOCAMONGE OF ACCUTACTOR	CTOCOACGE TTOCAMONGE OF ACCUTACTOR	TTGCANGAGE AACGITCTCG	90	COTCOCTACA	CHARCES	ATAGCCGAITT TATCGCCTAC	TCAGCCTTGC AGTCGGAACO	CTACGAACGC	CACCTATTCT	CACCIOCOCOCT
COCCAMONA ACCICATO COCCATOTAL DECENTATAL	COCCAMONA ACCICATO COCCATOTAL DECENTATAL	ACCIONATA	00	CTACCCTAC OCTCGGGTTG	CCGCCCCTCA	ACTTCTACCC TGAAGATCAG	CUTATTTRCC GCATAAACGG	OTOCCAGAGG CACGOTCTCC	TGCTFTGCCAC ACGAACGGTG	CTATCACATE GATACTETA: Forfiv
HYPPICCARA ACTOCRAGAT ACCCTATICE TIXTIGHSCEN ACCOUNTIES ARABAMACITY TORICHTOTA TOGOGATAGG ACGACAGGT TGACGTCASC	A ACTOCAAGAT ACCCTATCC TECCGTSCEA	ACCCTATCC TOGGGATAGG		TRCCGTTGCCA ACCGCACGGT	ACCGCAGCCA TGACGTCGGC	ACCCAGACAAG	CAGCTGGCCT TGCTGCAGGGGGTCCC	TREFFECTORING ACRECTED TO THE PROPERTY OF THE	CCCTCTCATA	CCTUNTATO: GGACTATAG

Figure 150

PMRKAdSgag MERGRZ

24201	CCTCOCTCAA	COAACTCCCA		AGASSTK TTKKS TCCCAGAAAG	ACGC/ACGACGACG	אאשרמאנאדאט (דונאנאנאבאנינר (CAAACGCTCT	CONTORCOTT	AACAGCGAAA ATGAAAGTCA TIOTCOCTIT TACTITCAGT	ATGNANCITCA TACT'FTCAGT
24301	cretoakoro	TTGGTGGAC TCGAGGTGA	TCGACGCTCA	CAACIRCERT	CTAGCCCTAC	TAMAACTACAG	CATCGAGGTC	ACCCACTITO	CCTACCCOCC	ACTTAACCTA TOAKTTGGAT
24401	CCCCCCAAOO	TCATGAGCAC	AGTEATGAGT	GARCTCATEG					AGAACAACA	CHICHICCON
24501	TACCCOCAGT	TOCCACGAG	CAGCTAGGG	CCACCCAAGT	AACGCGCGCTC		TRIGARIGACIO ACCTECTEGE	ACCCAMACTA	ATCATCCCCC TACTACCCCC	CAGT :CTCGT OTCACGAGGA
24601	TACCOTGGAG		TGCAGCGGTT ACGTCGCCAA	CTTTGCTAAC	CCGGAAAATGC GGCCTCTACG	ACCCATACT TCCCATCCA	AGACTIAAACA TCTCCTITGT	TTCCACTACA	CCFFFCGACA	GOCCTACGTA
24701	COCCAGOCCE	OCMOR	CAACOTOGAO GTTGCACCTC	CTCTGCAACC	TRATCTCCTA	CCTROOATT	TTGCACGAAA	ACCOCCTTOO TOOCOGAACC	OCAMACOTO COTTTTGCAC	CTICATTCCA
24801	CCCTCAAGGG		COAGGCCCCC COCGACTACG CCTCCGCGCG GCCTGATGC	TCCGCGACTG AGGCGCTGAC	CGTTTACTTA	TTTCTATGCT AAAGATACGA	ACACCTOGCA TYTOTTACCGT	GACGGCCATG	GGCGTTTGGC CCGCAAACCG	AGCAGTGCTT TCGTCACGAA
24901	GOAGGAGTGC CCTCCTCACG	AACCTCAAGG	AGETGENGAA	ACTRICTAAAG TGACRATTTC	CNAAACTTGA GTTTTGAACT		CHOCCHOAND		CCOMOCOOCO	OCACCTOSIC (CGTOGACCO!:
25001	GACATCATTT	TCCCCOAACO AGGGGCTTGC	CCTCCTTAAA	ACCCTFXCAAC TOXOACGTTG	MAKENETICC TEEENONCOO	AGACTTCACC TCTGAAGTGG	ACAGITICGE		GANATCCTTG	AAATAGGATU
25101	AGCGCTCAGG TCGCGAGTCC	ANTETTOCCC	GCCACCTOCT CGCTGCACGA	GTGCACTTCC CACGTGAAGG	TAGCCACTIT	CACGGGTANT	ACTACCCCCA TCATGGCGCT	Arcecteco	CCCCAAACCC	COCHUNCAT
25201	CCTTCTCCAC	CTAGGGAACT	ACCTTOCCTA TOGAACGGAT	CCACTCTVAC GOTGAGACTO	ATAATGGAAG TATTACCTTC	ACCITCIANCES TOTA TOCACITCICE ACT	CCCACAT	CTCGAGTOTC GACCTCACAG	ACTOTOCOCTO TGACAGCGAC	CAACCTATKIC GTTGFATALTI
25301	ACCCCGCACC	CONDONCEA	TECCAATTEG ACCTTAAGE	CACCTCCTTA	ACCANAGTCA TOCTTTCAGT	ANTIANCIATA ACCITITURGO TIANTAGCCA TOGRANCICO	ACCTTTGAGC TOGAAACTCG	TOCAGOOTICC ACOTCCCAGO		GANNAGTCCTI CTTTT/AGG"
25401	CONCINCION	CACTITION	ACTOCRODOC TGAGGCCCCG	TOTOGACOTO ACACCTOCAG	CCGNATGGAA	COCAMATTE	TACCTGAGGA	CHACCACIOCC	CACCAGATTA	CCARGATAT
25501	AGACCANTCC TCTOOTTAGG	COCCOCCTA	ATOCONACT TACCCTTCGA	TACCRICTUSC ATGGCGGAIT	CANTAATIANS CANTAATIANS	ACCCCCCTTTA	TCTTRACCAM AGAACCAGTT		TCAACAAAAC AGTIGTTICU	CECECOMON CARLESTATICAL
25601	TTTCTGCTAC	CHITCCCTGC	CCCCCAAATO	AACCTCCC AACCTCCCCC	ACTIC COCOCCA TO TICA COCOCCA T	CCTCGAGITIG	CCAMICCCC	תמכנת מכנה מכנת מכנה	CCCCATAGTC	GEOTOGO

Figure 15F

TETOCADACE TESTECTETO AGREGETE TODAGOCATT GGARETETGE AATTITATRA GRAGITIOTO ECATOGOTET ACTITAACE CITICIOGAA ADAGOTETOO AGENGAGAG TEGOCOCGAG ACETECOTAA CETTIAAGAGT TAAATAAC ECTEAAACAE GGINGEEAGA TGAAATTGGG GANGAGEECT

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Psfl

				IIS d	_ •					
25701	GOCCCTTOC	Trecendant	GCCACCCANA		AAGAAGCING NATINAYYANG	ההראהיההאהם	האכמאממאמ	AATACTGGGA	CARTCAGGCA	CARCACATETY
٠	CCCOOGAACO	AAGGGTCCTA	CCORMANITY	TTCTTCGACG	דדכידיבהאהם זרריאמאטימה	CONTRACENCE	CTISCICCITEC	TTATOACCCT	OTCACTCCOT	CTCCTCCN.
						1 tendili				
25801	TOCACCIACCA	GOAGGAAGA	ATCATCANO	ACTERCANGAN	נענבאינאיניאינ	מאאמנידדככני	AGGTCGAAGA	GGTGTCAGAC	GAMACACCUT	CACCITACIO
	ACCTGCTCCT	CCTCCTCCTO	TACTACCTTC	TGACCCTCTC	CONTINUES.	CITTEGANGOE	TCCAGCTTCT	CCACAGACTO	CTTTOTOCCA	OTGOTAGC: A
25901	COCATTOCCC	Teaceacae	CCCAGANATC	GCCAACCGGT	TECANICATION	CTACAACCTC	כמנזכניזכאם	0000000000	CACTGCCCOT	TOTACOGACCC
	OCCITARGOCCO	AGCGGCCUCG	GOTTETTING	CCOTTGGCCA	ADVITOTACC	CATCITTGCAG	CCGAGGAGTC	crececcecc	GTCACCOCCA	ACCOCATOR
26001	ACCOTAGAT	GOODCACCAC	TODANCCARR	OCCUGINAGE	CCAMICAGCC	GCCGCCCTTA	CCCCAAGAGC	AACAACAGCG	CCAAGOCTAC	COCTCATOG:
	TTOOCATCTA	ccercicona	ACCITICATIC	CONCATTON	GOTTCOTCOO	CRACARCANT	COCCINCICO	THETHOREGE	GGTTCCGATG	OCCIMOTACCO;
26101	OCCOOCACAA	GAACGCCATA	OFFICETIOCT	TOCANOACTO	TOCOGRECAAR	ATCTCCTTCG	CCCGCCCACT	TCTTCTCTAC	CATCACGACG	TOTAL PROFILE
	COCCCOTOTT	CTTCCCCTAT	CHACCHACCA	ACCETICIONO	ACCCCCGTTG	TAGAGGAAGC	GCCCCCCCAA	AGAMANGATO	GTAGTGCCGC	ACCTITIONACTO"
26201	CCUTAACATC	CTCCATTACT	ACCONCATOT	CTACAGCCCA	TACTRICACEG	CCCCCCAGCCG	CAGCHACAGC	ACCOROCCACA	CAGAAGCAAA	CKACKIACCOGA
	OCCATIGING	GACCTANTGA	TOCCAGTAGA	GATGTCGGGT	ATGACGTTGC	CACCOTOGCC	Greensnes	recessorer	arcricom	CCCCCCCCCT
26301	TAGCANGACT	CTGACAAAGC	CCARGALATC	CACAGCGGCG	GCAGCAGCAG	GACCIACIONOC	GCTGCGTCTG	GCCCCCAACO	AACCCGTATC	GACCCOCOAR
	ATCOTTCTOA	GACTOTITICO	GGTTCTTTAG	OTOTOCOCOC	COTCOTCOTC	CTCCTCCTCG	CONCOCAGAC	cacacantac	TTOOOCATAG	CTOGGCGCTC
26401	CTTADAAACA	GCATTITICS	CACTUTAT	CCTATATIFIC	AACAGAGCAG	GOCCANOA	CANGACCTOA	AAATAAAAA	CAGOTETETO	COATCCCTCA
	GAATCTFIGT	CCTAMAAAGG	GTGAGACATA	CCATATAAAG	TRACTORITE	CCCCCCTTCTT	GFTCTCGACF	TITATITIE	GICCAGAGAC	OCTAGOGART
26501	CCCCCAGCTO	CCTOTATICAC	AAAAGCGAAG	ATCARCTICG	GCGCACGCTG	האחתהפכפס	AGOCTECTE	CACTANATAC	TOCOCOCTOA	CTCTTAAGGG
	GOOCGTCGAC	CCACATAGTO	trincocrac	TAGTCCAAGC	CCCCTRXCTAC	CTTCTGCGCC	TECCHONONA	OTCATITATO	ACCCCCCACT (GAUAATTCC .
26601	CTAOTITICOC	occentricic	ANTITAAGC	GCGAAAACTA	CGTCATCTCC	ACICOCICCACA	CCCGGCGCCA	GCACCTOTTG	TCAGCGCCAT '	TATHINGCAN
	GATCAAAGCG	CCCCAAAGAG	TITAATICO	CCCTTTTGAT	CCACTAGAGG	reaccagnst	COCCCCCCC	CCTCCACAAC	ACTCCCCOTA 1	ATACTICOTITY
26701	DANATICCCA	COCCCTACAT	OTCCAGITIAC	CARCCACAAA	TGGGACTTCAC	GOCTGGAGCT	CCCCAAGACT		AATAMACTAC I	ATGARCOCOG
	CTTTAAGGGT	OCCOUNTGEN	CACCTCAATO	Grecongrer	ACCCTGAACO	ACCCTGAACO CCGACCTCGA	COCCUTICTGA	TCAGTTGGGC .	Trafffcato .	TACTCOCOCC
		Ecoftv			1 m	Econi				
26801	GACCCCACAT	DATATCCCOO	OTCAACGOAA	TACOCOCCCA	CCCIANACCCA	CCCAAACCCA ATTCTCTCTCG	AACAGGCGGC	TATTACCACC	ACACCTCOTA 1	ATMACCITTAN
	CHOOCOTICTA	CTATAGGGGCC	CAGTTGCCTT	ATGCGCGGGT	CACTITIOSCT	TANGROCACC	TTGTCCCCC	ATAATOOTGG '	TOTOGRACAT 1	TATTCCAATE
26901	TCCCCOTAOT	TOOCCCOCTG	CCCTOGTGTA	CCACCOAAAGT	CCCCCCCCCC	CCACTGTGGT	ACTITICE CAGA	GACGCCCAGG	CCOARGITICA (CANTIACTAN!
	AGGGGCATCA	ACCOGGCGAC	GGGACCACAT	GOTCCTTICA	GOCCACOCT	GUTGACACCA	TOAAGGGTCT	CTGCGGGTCC	OCCTICAMET (CTACTGATTO
27001	PCADDODCOC	AOCTTGCGGG	COCCITICGE	CACAGOOTTC	ומפובמככנה	CCAGGGTATA	ACTICACCTOR	CANTCAGAGG (CARCTCAACT
	AGreceded	TCGAACGCCC	GCCGNANGCA	OTOTOCCEACG	CCARCIGOCC	CCTCCCATAT	TOAGNGACT	GTTAGTETEC	CGCTCCATAA	Greanstra.
27101	ACGAOTCOOP	GAGCICCTCO	CHROSTICAC	GTCCGGACGG	GACATTICAG	ATCGGCGGCG	centeener	-		CANTOCTANG
	TGCTCAGCCA	CTCGAGGAGC	DAACCACACACC	CAGGCCTGCC	CTGTAAAGTC	א מככנעכנעכ	GCCCGGCGAG 1	AAGTAAGTGC (GGAGCAGTCC G	GITAGGATIG

· Figure 1562

				- Company	ACCUCATANA	CCALTRONACO (GACCACCTACG	ACTOLATOTT	AAGTOOAGAG	CCAGACCAAC
27301	CCICCCOOCC	MCTATCCUBA	TCANTTIAL					TUNCTTACA	TICACCTUTC	בטדלידונט
	CONCOCCO	TCATAGGCCT	AGITANIA				_	CTPREAMEN	CCCCACCATC	ATATECAGES
27401	TOCOCCTGAA		CACTGACAC	CCCACAACTG	CATHALLAN.			GARACTTANC	OGGCTCCTAG	TATACCHC"
	ACCCCOACTT	TUTCHECAG	פופאראוניאני	1000			_	COCCCCTOC	TACTFICACC	CICACACIOS:A
27501	CCCOGCGCAC	000010000	TACCGCCCA	COCAGACT	CLCCO-MCG.			CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	ATCAACTCOC	ectoreco."
	ومدرودواه	CCC AGGCCG	10000 N			ngn				
276.01	ومعريانتيانيان	TCACTOTICAL	TITISCARCTOT	CCTAACCCTG	GATTACATCA	136	_	GTOCHWOTA	TATAAATAC	AGARATTA: A
	GGGACACANG		AACGITICACA	COATTREGAC	CTAATGTAGT	TCTAGANCA	ACCCTAGAGA	CACGACTCAT	ATTATTATO	TCTTTANT
27701	ATATACTOO	-	CCATCCTGTA	AACTICCACCO	TrimeAcces	CCCAAGCAAA	CCANGGGAA	CCTTACCTOO	TACTITIONS	ATCTCTC
•	TATATOACCC	_	GGTAGGACAT	THECOOPOSC	AGANGTGGGC	GOOTTCOTT	CCHICCOCH	CCAATCCACC	ATGAGATTO	ואמארטערער
27801	CHOTOATTEA	CAACAGTETC	AACCCAGACG	CARCITOAGTET	ACCIACIACIANC	CICICCONOC	TCARCTACTC	CATCAGAAAA	AACACCACCC	TCCTTACCT
	CACACTACA	_	TTOOCHCTCC	CTCACTCAGA	TIGGRETICITIO	GAGAGGCTCG	AGTCGATCAG	GIAGICITAT	norcence	ACCOUNT IN THE
27901	CCGGGGACGT		CACCOOCCOC	TYCACCACAC	CTACCGCCTG	ACCOUNTACC	AGACTETET	COCACAGACC	TCAATAACTC	TOTTTACCAN
•	GOCCCTTOCA	Ξ.	GTCCCCCCC	ACOTOGRAND	CATCCCCCAC	TCCCATTIOG	TCTGANAAAG	BECTETETO	DADITATION	
28001	ABCACACACT	GAGCTTAGAA	MCCCTTAGG	GFATTAGGCC	MANGEGERAG	CTACTGRAND	GITTATGAAC	ANTICAROCA	ACTICTACOGO	-
	TIGHTCTTCCA	_	TTOOONATCC	CATAATCCCIG	Tricceconc	CATCACACCC	CAANTINCTITO	TIMAGEICGE	TOMONTOCCC	GATARCAT.
		Xtbal								
10186	TO STATE OF THE PARTY.	CTAGAATEGO	COPPOSEDIT	ATTETETET	THETOATICE	CTITATICIT	ATACTAACOC	TICHCHOCCT	MOOCICOCC	
10107			CCACCCCAA	TAAGAGACAG	AACACTANGA	GANNTARGAA	TATEATTECO	ANGREGOR	TICCGAGCG	
10101	TO STATE OF THE ST		CAGCTTTTTA	AACGCTGGGG	TCACCACCCA	NGATGATTAG	GTACATAATC	CTAGOTITAC	TCACCCTTOC	
10907		_	GTCGAAAAAT	TIGGGACCCC	AGCGGTGCCT	TCTACTAATC	CATCTATTAG	CATCCAAATO	AGTOGGRACO	CAGILLAGE
	Kpril								THE PARTY.	BITATAGN .
28301	COTACCACC	. AAAAOOTOGA	TTTTANGGAG	-	ATCHTACATI	COCAGCTGAA	CCTANTAGE	CCACCACICE CCACCACICE	Pre-	
	CCATGGTGGG	3 TTITICCACCT	AMATTICCTC	GOTCGCACAT	TACANTGTAA	CCOTCGACTT	CCATTACTCA	Corosionen	Carrier Park	-
28401	ATGAAAAGCT	r ochrythcoc	CACAAAAACA	NAATTOCCAA	GTATICITY	TATCCTATT	UTICAGCCAOG	TCACACTACA	CACIAIMA	
	TACTITICAR		GIGITATION	TTTANCCOTT	CATACGACAA	ATACCATAMA	cconcoence	ACTIGICATION	20101010	-
			Hsil	1107						W. J. L.
SASDI	CLANCETAAA	A ACTICATABLA	CHITTATOTA	TACTITICCA	TITTATGAM	TOTOGOGACAT	TACCATGTAC	ATCACCACAC	AUTHING!	
40003			GAAAATACAT	ATCAMAGGT	ANATACTIF	ACACCCTGTA	ATCCTACATG	TACTOUTUR	ורשואוורים	
28601	CAAAATTGTG	_	TOTACHET	POCTOCACTO	CTATGCTAAT	TACAGTECTE	CETTINGICT	OTACCCTACT	CTATATTAA	ATGESTATES .
	OFFITANCIAC	_	ACCOTOMAG	ACGACCTCAC	CATACCATTA	ATCTCACGNG	CCAAACCAGA	CATGOOALGA		
10787	CACCACACT	P TATTGAGGAA	AKHANATOC	-	-	ACCTABILITY	ACCACTAACT	CCTTTMC1CO	CHECKTOCK	STOTETAN:T
	CTOCOTOGAA		THETHTAGG	•	-	FCCATTACMS	אנאיאיאין ועי	A Park Property of	SECTION OF SECTION OF	
28801	ANNOTINGE	_	_	-	OCTUATTUC CENCENA POC	ACCACTCANTAC	GTAAGGGGAC	TTOTTANCTO	AGATACACCC	
	TTTCATCO	O TANTATTAAT	בייועדכרי אי	ATTIGORA						

Figure ISR

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. 20901	OCCUTACANC CRECATEME	CITGAAGTCA	CCGMGGACC	ATCTCACACAT	CACTGAAACC	CCAGCACCTO	PCCCGCGGAT AGGCGCGCTA	PTCPTCCAGT AACAAGGTCA	CCAACTACAA GCTTGATGTC	CCACCCACTC GCT NGT 311
29001	TAACAGAGAT					TETACCACAA	ATACACCCCA	ACTITICISCE TCAMOACCG	TTTOTCAATA	ACTOGGATINA
29101	CHICOCCATO	10010GTTCT ACCACCAAGA	CCATAGCCCT	TATTTTTTA	TCH:CTTATTA ACCOUNTANT	TTATCHYNCT ANTACACYON	CATCTCCTGC	CTAMAGGGCA	AACOCGCCCC	ACCACCCATC TOGTH (CF: PAG
29201	TATROTCCCA ATATCAGGGT	TCATTOTCCT AGTAACACGA Xhol	ACACCCAAAC TOTORGITTIO	AATTATTETAA TTACTACCIT	TCCATAGATT AGGTATCTAA	GENERALATION CONTROLLE	AAACACATGT	TCTTFICTCT RGAAAAGAGA	Tacagtatga Atgicatact	TTAMATCAUA AATTTACTCT
29301	CATGATTCCT . GTACTAAGGA	CATCATACCT CCACTATTTA GTACTAAGGA GCTCAAAAT	TATTACTOAC ATAATGACTO	CCTTGTTGCG		CTTTTTTTTTT CONSCICCAC GAAAAAACAC GCACGAGGTTS PSI	ATTOGCTOCO TAACCGACOC	OTTPCTCACA CAAAGAGTOT	OTTICICACA TCOMOTAGA CAAGAGIOF AGCITICATCT	CTGCATTC A GACGTAAG .F.
29401	OCCTTCACAO COGAAOTOTC			TTACGGATTT OTCACCCTCA AATCCCTAAA CACTGGGGGT	COCTCATCIO CAGCCICATE GCGAGTAGAC GICKRAGTAG	CACCTCATC	ACTISTICATEA TEGECITITAT TGACACEAST AGEOGAAATA ESSP		CCAMBCATT	GACTOOOTH
29501	CACACGCGAA	TOCATATOTIC ACCTATAGAG	AGACACCATC TCTGTGGGTAG	CCCAOTACAG	GGACAGGACT	ATAGCTOAGC TATCGACTCG	TICTITAGIAT TCTTTAATTA	TCTTTAATTA	TOAAATTTAC ACTITTAAATO	TOTOACTTT
29601	CTCCTCATTA		ATCTUCGITT	TOTTCCCCOA	CCTCCAAGCC	TCAAAGACAT AGTITCTGTA	4 F	GATTCACTCG	TATATOGAAT ATATACCTTA	ATTCCAAGI T TAAGGTTCAA
29701	CCTACAATGA	AAAAAGCGAT	CTTTCCGAAG	CCTCGTTATA	TCCAATCATC	TCTGTTATCG AGACAATACC	TOPICTISCAD TACCATCTTA ACARGACGTC ATOGTAGAAT	TACCATCTTA	OCCCTAGCTA CGOGATCGAT	TATATCCC 1 A ATATAGGAT
29801	CCTTONCATT			CATGAACCAC GTACTTGGTG	CCAACTITICC GOTTGAAAGG	محدودودور محدودودور	TATGCTTCCA ATACGAAGGT	CTGCAACAAG GACGTTOTTC	TTGTTGCCGG AACAACGGCC Xb	CCCCANACA I CCCCANACA I
29901	CCAGCCANTC	AGCTCGCC	ACCITICACCC	ACCCCCACTG	AAATCAGCTA	CTTTAATCTA	ACAIRIARRAG. ATGACTGACA TOTCCTCCTC TACTGACTGT		ECCTAGATCT AGAA	AGAAATRGAC
30001	CCTTAATAAT	CAGAGCAGCO	CCTGCTAGAA	AGACGCARRO TETOCOTECE	CAGCGGCCGA	COTTOTOCO	ATGAATCAAG TACTTAGTTC	AGCTCCANDA TCGAGGTTCT	CATOOTTAAC	TTOCACCAGT AACTTTOGTCA
30101	COTTTCCCC	TATCHITGT ATAGAMAACA	CTCCTAAAAC	AGGCCANAGE TCCGGTTTCA	CACCTACGAC	AGTAATACCA TCATTATTGT	CCGGACACCG	CCTTAGCTAC	AAGTTGCCAA TTCAACGGTT	CCANGCOTI''' GGTTT:(IFAL:T
30201	CANATIOONS CATTANCCAC	GPCATGGTGG CAGTACCACC	CACIMANGCC CTCTTTTCGG	CATTACCATA GTAATAGTAT Balli	ACTENGEACT TRAGICGIEA	CCCNTCTTIG	CCANGGCTGC	ATTCACTCAC	CTTOTCAAGO	ACCTGARGAT TOGACTCCTA
30301	CTCTOCACCC	CTCTOCACCC TTATTANGAC CCTGTGCGGT CTCAMGATT TAATCCCTT TAACTMATAA AAAAAAAA TAAGGAGGGGG AATAATCTG GGACACGCCA GAGTTTCTAA AATAAGGAAA ATTGGTAGT	CCTOTOCOOT	TENTIANGAC CCTUTACOGT CTCANGATT: TRATECECTT TAACTAATAA	TTATTCCCTT	TAACTMATAA	ANAMANTA THANGCATCA TITITITITATE ATTITITITATE		CTTACTTAAA	NTCAGTTAGG TAGTCAATCG

рмкклабану непбя2

30401	AAATTICTUT	CCAGTITATE	CAGCAGCACC	nectioners Aggregated	CCTCCCACACT	CHATTATTGC	AGCTTCCTCC	TOGCTOCAAA ACCOACOTTT	CHITCICCAC	ANTCTAAATO
30501		_		CCPTACCCAC	TATCTTCATG	THEFTER AGA	TGAAGCGCGC	AACACCOTCT	GAAGATACCT	TCAACCCC(:T
30601	GTATCCATAT		CCGGTCCTCC	AACTESTICECT TTGACACGGA	TTTCTTACTC AAAGAATGAG	CHCCCTFTCT	ATCCCCAAT	OCCHANGENCE CCCANAGENC	AGAGITCCCCC TCTCAGGGGG	TOGGOTACT:
30701	TCTTTGCGCC	TATCCGAACC	TCTANTTACC ACATCAATEST	TCCAATGCA TGCTTGCCT	TKICTTRACECT	CAMMATCAGE	AACTOCCACAGA	CTCTGGACGA	OCCOOCANC CCOOCCOTTO	CTTACCTCCY: GANTOCAG: 1
30801	AAAATOTAAC				CAGTETICTAL	MACCHORAM	TATCTOCACC	CCTCACAGTT	ACCTCAGAAG TOGAGTCTTC	CCCTANCTAIT
30901	GOCTOCCOCC CCGACGGCGG		TOGTCCCCCC	CAACACACTC	ACCATOCAAT	CACAROCCCC	CCATTOCCAC	CACGACTCCA	AACTTAGGAT	TOCCACCCAA ACOGTOOGT
31001	CCTOGGGROT		AGGAAAGCTA TCCTTTCGAT	CCCCTCCAAA	CATCAGGCCC	CCTCACCACC	ACCGÁTAGCA TGGCTATCGT	GTACCCTTAC CATGGGAANG	TATCACTOCC	TCACCCCCTT AGTOGGGGA1
31101	TAACTACTOC		THOOOCATTO	ACTTORANGA TOANCTTTCT	COCCATTTAT	ACACAMANTO	GANACTAGG	ACTAANOTAC TGATITICATO	OCCCUAOCA	TOCHTOTAL . ACCTACATIVE
31201				TOOTECAGOT ACCAGOTECA	CACTGATATTA	ATAATACTTC	CTTGCAAACT	ANGITACTO	GAGCCTTOOG	TITICATICA MANCTAN '
31301	CAUCICAATA		16TAGCAGGA ACATCGTCCT	CCTGATTCCT	THUNTTETEN	MACAGACGC	CTTATACTTG	ATOTTACTTA	SCCOTTTOAT AGGCAAACTA	GCTCANAACE COAGTFFT
31401		•	CAGGGCCCTC	TTTTATANA ANAANTATTE	CTCAGCCCAC GAGTCGGGTG	AACTTOGATA	TTAACTACAA AATTGATGTT	CANAGOCETT	TACTTOTTTA ATGAACAAAT	CAGCTTCAA \
31501	CAATTCCAAA	AAGCTTGAGG	TTAACCTAAG	CACTGCCANG	CCCAACTACA	TTGACGCTAC	AGCCATAGCC TCGCTATCGG	ATTAATGCAG TAATTACGTC	GAGATOGGCT	TOMATHOUSE ACTIVANCEA
31601	TCACCTAATG			AAAACINAAA	THRRICCATOR	CCTAGAATIT	GATTCAAACA	AGGCTATGGT TCCGATACCA	TCCTAAACTA AGGATTTGAT	CCTYGACCTR
31701	TTAGTTTTGA		GCCATTACAG	TAGGAAACAA	ANATANTOAT TTTATTACTA	MACTAACTE	NEACCTRATES	ACCAGETECA TOCTEGAGET	TCTCCTAACT ACACCOATTICA	GTAGACTAAA CATCTGATTT
31801	TECAGAGAAA	CTACCATITIO	TCACTTTGGT AGTGAAACCA	CTTACAUA	TGTCXXCAGTC ACACCGTCAG	AAATACTTGC	TACAGITICA	CAAAACCGAC	TTAAAGGCAG	MACCCAGGT
31901	AFAICTCOAA	CAGTICANG	TOCTCATCTT ACCAGINGAA	ATTATAGAT TAATATTETA	TTCACCAAAA	TEXAGECTA ACCTCACCAT	CTANACNATT	CCTTCCTGGA	CCCAGAATAT	ACCTTCAAAT
32001	DANATOGNA T	GANATGANA TETTACTUM CTITACETET AGANTGACTT	OCCATCACCT	ATACAAACGC TATGTTTKCG	TGTTGGATTT	AFFECTANCE	TATCAGCTTA	TCCAAAMICT AGGTTTTAGA	CACCOTANAA	CTCCCAAAAG

Figure 15T

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32101	TAACATTOTC	ACTICARCITY TCACA	TOANTITICE	TCTOTTTTTO	AAACCTTCITAA TTTKX:ACAIT	CACTANCEAT	TACACTAAAC	CCATCTGTCC	MANCAGGAGA	CACAACTC:A GTGTTRACA:"
32201	ACTOCATACT	CTATOTCATT	THE ATTROCATE AND TACKETS	ACCAMACICA	ACAM TACAT TOTTVATOTA	TATTGAMATA	PPICATCACAT NACCAPTUTA	CCTCTTACAC GCAGAATGTG	TETTTCATAC AAAAAGTATO	ATTRECECAN: TAACRICITY:
32301	AATAAAGAAT	CCATTOTCTT	ATCITITCANC TACAMGITIG	CACAMITAN	TTCAATTACA AAGTFAACGT	GAMANTITICA	NTICATE TITE TENTE ANNA	CATTCAGTAG	TATAGCCCCA	CCACCACATA GENERALIA I
32401	CCAATATOTC	ATCACCOTAC TACTGGCATO	CTTAATCAAA	CTCACACAAC	CCTAGTATTC	AACCTVACAC	CTCCCTCCCA	ACACACACASAG TGTUTGTCTC	TACACAGTCC ATGTGTCAGG	AAAGAGGG
32501	GCTGGCCTTA CGACCGGAAT	ANAGCATCA	TATCATGGGT ATAGTACCCA	AACAGACATA	TTCTTMSGTG		TTATATTCCA CACKOTTTCC ANTATARGOT OFFICEAAAG	TGTCGAGGCA ACAGCTCGGT	AACGCTCATC	AGTOATATI .
32601	ATABACTCCC TATTIGAGGG	COGOCAGCTC	ACTTANGTTC TOAATTCAAG	ATGTCCCTOT	CCAN KTROCTO CKTCCACCAC		CAGOTT	CTTGCGGTTG	CTTAACGGGC GAATTGCCCG	CCCCTTCCT.
32701	AAGTCCACOC TTCAGOTGCO PMI	CTACATGGGG GATGTACCCC	GT AGAGTCAT CATCTCAGTA	AATCCTCCAT	CAGGATAGGG	CONTROPOCT	GCAGCAGCGC	GCGAATAAAC C(XCTTATTTG	TOCTOCCOCC ACGACGGCGG	GCCACACCA CGGCGAGGCA
32801	CCTGCAGGAA	TACAACATOO ATOTTOTACC	CAGTOGICTC GTCACCAGAG Pnfl		ATTCCCACCO TAAGCGTAAC	CTCARCATA ATTOCCACCO CCCGANGAT ANABCIACCTT GAGTGGCTAC TANGGGTTATC GAGGGTCGTA TYCCGCGGAA		OTCOTOCOGO CAGGAGGCCC	CACAGCAGCG GTOTCGTCGC	CACCCITOAT .
32901	ACTCATTANAT	CAGCACAGTA	Achocadeac	ACCACCACAA		TATITITICAN ANICCCACAG	TGCAAGGCGC	TOTATCCAAA	GCTCATGGCG CGNGTACCGC	CCCTGOTCTC
33001	AACCCACOTO TTGGGTGCAC	GCCATCATAC CGGTAGTATG Kprf	CACAAGCGCA	GCTAGATTAA CCATCTAATT	GTV4GCGACCC CACCGCTGGG	CTCATABACA GAGTATTTGT	CCCTCGACAT	ANACATTACC TITOTAATOB	AGNAAACCOT	TOTTICTAATT ACAACATTAA Pedi
33101	CACCACCTCC GTGGTGGAGG	CCCATGOTAT		ATTAAACATO TAATTTGTAC	GCGCCATCCA	CCACCATCCT	NACCAGETO PTTOSTEGAG	GCCAAAACCT COCTTTTGGA Engfly	מכנכסכנס נמסכנפננמ	tatacactu». Atatotogo:
33201	AGGGAACCGG TCCCTTGGCC	CACACCTION	ATGACAGITAG TACTGTCACC	AGAGCCCAGG TCTCGGGTCC	ACTEST PARCE TOACCATTOS	ATCGATCATC TACCTAGTAG	ANGCTCGTCA TACCAGCAGT	TCATATCAAT	GTTGGCACA	CACAGOCACA GRUTTCOTOT
33301	COTOCATACA	CTTCCTCAGG GAAGGAGTCC	ATTACAMCT		TACANCCATA ATCTINGTAT					CACTGCAGAG GTGACGTCC
33501	ANGACETICA TTCTGGNGCG GGNGGTNGAC CCTCCATCTG	ACCEPTOROT TOCATTOROT GATCCCTACT CTAGOGIATOR	CCANCALOTA OCANCALOTA GTACOLAGIO CATICCTCAC	HOTENAMETO ACAGTETICAC CACCOMINCA GCGGCTCTGT	TTACATTCOS ANTOTAAGCC ACCCACATCG TOGCTCTAGC	CETACE RECEST CETACES	ATCATCTCC I TACTAGERACO AGTICTCATIC TCACAGERACO	AOTATOGRAG TCATACCATC CAAATGGAAC GTTTACCTTG	CCCCCCAAAG GCCCCACOTA CCCCCTCCAT	TOTCTCANA ACAGAGETET GTCATATET CAGTATAAAVI

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וחאנו	CHURACTERA	Acreography	CHECKTRACAA	ACASATCTVAC	מוניותכנינות	TYTICICOCPTA	GATCCCTCTC		GTACTATATC	CACTCTCTA	
7000	CHOROCOM	TOTACCACOC					CTANCANANC	ACATCATCAA	CATCATATAG	CTCACAGALT	
11701	PAGE TROUBE					מינגינענינני	TYSATAACATC	CACCACCGCA	GANTARGCCA	CACCCAGCC/	
4016	Tremplant	CCCCCCCCCCAC				COCCCACGOG	ACTATTOTAG	OTCOTOCCOT	CTTATICOGE	GTCC(:1CO.H	
11001	B.C. B.C.B.C.B.C.B.C.	The state of the s			CACAACACETTC	CANCANCCAT	CHARTERIA	TTATTCCAAA	AGATTATECA	ANACC'TI 'NNA	
	TCCATCTCTA	ACCAAGACOC			CUTTUTURAL	CTICTIOGTA	CAMANAAA	AATAAGGTIT	TCTAATAGGT	TITICONCILLE	
	Bom										
11001	ATTABGATCT	ATTAACTGAA	COCOCIECCE	PCCGGTGGCG	TYGTCANACT	CTACAGCCAA	AGAACAGATA	ATCOCATTO		CACANTOOCT	
1000	TACTTCTAGA			ACCCARCCC		GATCTCGGFT	TCTTGTCTAT	TACCGTANAC		GTOTT'ACCC!	
14001	TECANANGE	AACOCCCT		TOGACGTAAA	CCC TWANTED	TTCAGOGTGA	ATCTCCTCTA	TAMACATTCC		ACCATOCCCA	
	AGGILLICCO	THICCCOOR	-	ACCTOCATT	CCCATTTCCC	ANGTECERET	TAGAGGAGAT	ATTIOTANGO	TCGTGGAAGT	TOGTACOOCT	
34101	AATAATICIC	ATCTCOCCAC	CFTCTCANTA	TATCTCTANG	CANATCCCGA	ATATTAAGTC	CCCCCATTGT	AAAAATCTGC		CCTCCACCTT	
	TTATTARGAG	TAGAGCGGTG	GAAGAGITTAT	ATAGAGATTC	GTTTMOOGCT	TATAATTCAG	OCCUBITANCA	THITTAGACG		GCAGCTCOAN	
34201	CACCTCAG	CAGCGAATCA	TOATTOCAAA	AATTCAGGTT	CCTCACAGAC	CHCTATARGA	TTCAMAGCO	GAACATTAAC	AAAAATACCO	CONTICLEOR	
	OPCOGROTTIC	OPCCCTTAGE	ACTAACCITY	TTAAGTCCAA	GOAGIOTOTO	GACATATTCT	MOTHERCOC	CHIOFAATTO	-	GCTACKACAT	
34301	CORECETION	CAGGGCCAGC	TGAACATAAT	COTOCAGGIC	TOCACOGACC	AGCGCGCCA	CTTCCCCGCC	ACCAACCATG	ACANAGAAC	CCACACTCAT	
	CCADGGAAGC	grecedence	ACTIGITATION	GCACCTTCCAG	ACC/TOCC/TOO	TCCCCCCOT	GAAGGGGCGG	TCCTTCCTAC	STEEL ST	GGIGIGIGALIA .	
					Hindie						
14401	TATRACACC	ATACTCOOM	ATACTOGGAG COTATOCTANO	CACCCTAGCC	CCCATCTAAG	CTRGFTGCAT	GGGCGGCGAT	ATHARATOCA		CAMANATO	
	ATACTOTOCO	TATORGCCTC	GATACGATTG	orcecatedo	COCTACATTC	GAACAACGTA	CCCGCCGCTA	TATTITIACOT		יטאזניזירוט	
14501	CHETAAACICCT	COCCCAAAAA	ACIANGCACA	TCOTAGTCAT	CKTKATGCAG	NTANARGENG	GTAAGCTCCG	GNACCACCAC		ACCAPITITIO	
	CCGTTTCOCA	OCOCULTATA		ACCATCAGTA	CCAGTACGTC	TATHECOME	CATTCGAGGC	CHAMOROPIO		Total Automates	
14601	TCTCAAACAT	OPCTOCOOOF	TICTOCATAA	ACACAMATA	ANATAACANA	ANANCATTTA	MCATTAGAA	OCCIOICITA	-	MACAMELET !	
	AGAGITTOTA	CAGACGCCCA	AAGACGTATT	TOTOTITAL	TEATIGETY	TITICINANT	TTGTVATCT	COCHCHOMAT	-	I I ST I GOOM	
34701	ATANGCATAA	GACCONCTAC	CCCCATCCCO	OCCUBACCON	ANAMANACTO	GICACCOTCA	TTWAMACA	CCACCCACAG	CHECKEGOIC	TACAGGCTC	
	TATTCOTATE	CTOCCTOATO	CCCCTACGCC		TEFFITTEAC	CAGTGGCACT	AATTTTCGT	CONCENSION OF THE PARTY OF THE	-	CENTRUCTNO	
34801	TCATAATOTA	AGACTCOOTA		-	ATCONTCAGE	GCTANNAGC	GACCGAAATA	(ACCUCACO)	TATOTATO	CGTCCCCATC	
	AGTATTACAT	· retrandeent	THUTCTAOTC	_	TACCCAGTCA	CANTITICO	CIOCCITINE	**************************************	CONTRACTOR	AATACACCC	
34901	AGACAACATT	ACAGCCCCCA	-	_	NTAGGAGAGA	ANACACATA	AACACCTION	THE STATE OF THE S		TTATCOROGG	
	ACTOTIOTA	1 torcoood	ATCCTCCATA	-	TATECTETE	TELLGIGIAL	TIGICALIT	1		CACTOGACN:	
35001	PCCGGCTCCA	GANCANCATA	_		CCATAACAGT	CACCUTACC	ACATATETE TO	TTTTCCATA		GRIAGCTOTS	
	Accordant	CFICFICIAL	_			041444	CALABAAA	THERETTARCO	CHAMOTEC	ACAAAAARA	
35101	COCACCAGCT	CMICHOTCA	CAGTGTAMA	ANODICCANO	ACGRETCICT	CATATATATC	CTCAPTEITT	ACTOCATTOC	-	TOTTTTTOT	
	CCCHOCHCOA	Griner				CACAACTTCC	TCAAATCGTC	ACTICCOTT	TCCCACGITA		
35201	CCCAGAAAAC	COCACOCOUNT COCOUNT	r ceracecer	CTTTCCTTC		CICITICAAGO	AGTTTARCAG	TGAAGGCAAA	ACCOTOCANT	OCAOTICAAGG	

Figure 15V

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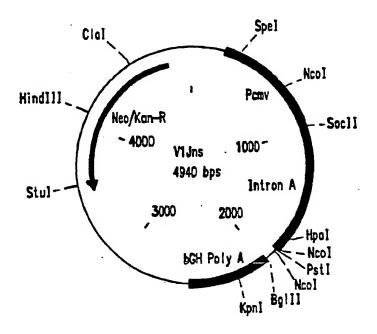
35301	CATTITARDA	AAACTACAAT	TCCCAACACA	CANTINADA AACTACAAT ICCCAACACA TACAAGITAC ICCARCCTAA AACCIACATC ACCGGCCCCG ITCCCAAGC CCGCGCCACG ITACAAACTA	Trencetan	AACCTACCTC	ACCOGCCCC	TTCCCMCGC	CCOCCCCACG	TCACAMCTC
	OTAMATHCP	TITICATOTIA	AGGGTTGTGT	ATCTTCAATG	ARCCERTANT	TTCGATOCAG	TTGGATGCAG TGAGCGGGGC	MODERACO	OCCOCC016C	ACTICITITIONS
•						Pd	orenees Eastle			•
•							Andrewson and a second			•
35401	CACCCCCTCA	-	GOCTTCAATC	CANATANGG	TATATTATT	ATCATCTTAA	TIMENATIC	OGATICTICODA	COCCADOCTO	DATEOCCETE.
	GTGGGGGAGT	. AATAGTATAA	CCGMUTTAG	GITTITATICC	ATATAATAAC	TACTACAATT	AATTCTTAAG	CCTACACCCT	OCCUTCCGAC	CTACCODANG
35501	CCCATTATOA	ו דוכדיוכוכככ	Trecoccocc	ATCOCKATIK	CCCCCTTCCA	(PECATOC'10)	TECHOGENGO	TAGATCIACGA	CCATCAGGGA	CACCTTCAND
	OCCUPANTACT	. AAGAAGAGCG	AAGGCCGCCG	TAGCCCTACG	COCOCIMICAT	CCCCTACGAC	AGGICCGICC	ATCTACTOCT	OCTAGNICCET	GREGARGITIC
35601	CCCACCAAAA	GOCCAGGAAC	CUTAAAAA	CCCCOTTGCT	GCCGTTTTTC	CATAGRETEC	GCCCCCTOA	CONGCATCAC	ANAMATECIAC	OCTCAAGTC/.
	COUNCOTHE	· ccoorcerro	OCATTITITE	GOTOCAACGA	CCCCAAAAG	CTATCCCACG	CCCCCCACT	OCTOGTAGTO	TTTTAGCTO	CONDITIONOR
35701	CACOTOCCOA	AACCCCAACAG	GACTATAAAG	NTACCAGGCO	PPPCCCCTG	CAACCTCCCT	CCHOCOCICT	CCTUTTCCGA	CCCTOCCOCT	TACCODATAC
	CTCCACCOCT	* tridocriore	CIGATATITE	TATRGTCCOC	AAAGCCCCAC	CTTCGAGGGA	CCACCCCAGA	COACAAGGCT	GCTACGGCGA	ATOCCCTATE:
35801	CTOTCCOCCT	TICTCCCTIC	GOGAAGCHTO	acactinete	ATARCTCACO	CTOTACGUAT	CTCANTTCGG	TOTAGOTCOF	TCCCTCCAAO	כיונאמניונים.
	CACAGGCGGA	ANDROGGANG	CCCTTCGCAC	COCCOANACING	TATCANGROC	GACATCCATA	GARTICANGE	ACATCCAGCA	AGCGAGGTTC	DACCCGACM.
35901	TOCACOAACC	: CCCCGFFCAG	CCCOACCOCT	GCCCCTTATC	COGTANCTAT	CENCTIGACE	CCAACCCGGF	AAGACACGAC	TTATCOCCAC	TOOCAGCAGY.
	ACCTOCITION	GOOGLANGTO	OGGCTGGCCA	COCOGNATAG	OCCAPTICATA	CCAGNACTICA	GCTTOGGCCA	Trefere	AATAGCCCTC	ACCGICCOTC
36001	CACTOOTAAC	. AGGATTAGCA	GAGCGAGGTA	TOTAGGCGOT	CCTACACACACT	TETTGAAGTO	STOCCCTARC	TACOGCTACA	CTAGANGGAC	AGFATTIÓOF
	OTOACCATTO	I TECTAATEGE	CTCGCTCCAT	ACATECGCCA	CONTENCTOR	AGNACTITCAC	CACCOGATTO	ATOCCOATOT	DATETTECTO	TCATAAACCA
36101	ATCTOCGCTC	: TOCTOMOCC	AGTTACCTTC	CONMANACAG	TICKETAGETIC	Trianceac	ANACAAACCA		COORGOTTIT	THEFTING
	TAGACGCGAG	ACCACTTCGG	fcantagang	commence	NACCATCGAG	AACTAGGCCG	Tricitrogr	OCCOACCATC	OCCACCAMA	ANACHARCTT
36201	AGCAGCAGAT	TACGCGCAGA	AAANANGGAT	CTCAAGAAGA	TCCTTTGATC	THITCTACGO	GENETICACIO	TCAGTOGNAC	DANAACTICAC	GTTANCCCA 11
	ACUTCOTOTA	ATOCOCONCT	TTTTTCCTA	GAGITCTICT	ACCIMACTAG	ANAGATECE	CCAGACTIOCO	AGTCACCTTO	CTTTTGAGTO	CAAFICCCTA
36301	THUGHCATO	AGATTATCAA	AAAGGATCTT	CACCTAGATC	CITTIAMATC	AATCTAANGT	ATATATOAGT	AAACTTGGTC	TOACAOTTAC	CNATCCTTA
	AAACCAGTAC	TETAATAGET	THICCTAGA	GTCCATCTAG	GAMATITAG	TTAGATTTCA	TATATACTCA	THTCANCCAG	ACTOTCAATO	CTTACCAMATA
36401	TCAGTGAGGC	ACCTATCTCA	OCCURACTOTIC	TATTICGITE	ATCCATAGET	OCCITOACTEC	CCOTCOTOTA	GATARCTACG	ATRCOGGAGG	OCTTACCATY:
	AGTCACTCCO	TOGATAGAGE	CGCTAGACAG	ATMANGENAG	TACCTATCAA	CCGNCTGNGG	GGCAGCACAT	CTATTGATGC	TATOCCCTCC	CCANTOGIAG
36501	TOOCCCCAOT	CETOCANTOA	TACCOCCAGA	CCCACCCTCA	CCGGCTCCAG	ATTTATCAGE	ANTANACCAG	CCACCCCCAA	GAGCCOAGCG	CACAMOTOGT
	ACCOGGGTCA	_	ATGGCGCTCT	OCCIOCGAGT	CCCCCAGGTC	TAMATAGECO	TTATTTGGTC	GOTCOCCTT	CCCOGCICGC	GICTICACCA
36601	CCTGCACTT	PATECOCCIC	CATCCAOTCT	ATTAATTCIT	accondinac	TAMATANGE	AGITCOCCAG	TTAATAGTTT	GCGCAACGTT	GITCCCATTG
	COACOTTOAA	_	-	TAATTAACAA	COCCCITICS	ATCTCATTCA	TEANGEOUTE	ANTTATCAMA	COCOTTOCAA	CANCOCTAAC
36701	CTACAGGCAT	COTOGRATICA	COCTOOLCOT	TROOTATEGE	TTCATTCAGC	Techoritee	AACCATCAAG	GCCAOTTACA '	TGATCCCCCA	TOTTOTACAA
	GATOTCCOTA	_	OCCAGCAGGA	ANCCATACCO	AAGTAAGTCO	ACCCANGCG	TTCCTACTIC	COCTCAATOT	ACTAGGGGGT	ACAACACGTT
			2	_}						
36801	AAAAGCOGTT	ACCIOCITICO	Greenecoar	Greenecoar correspond	ACTAACTING	CCCCAGTGTT ATCACTCATG	NTCACTONTO	OTTATOOCAG (TICACTTACT
	TTTTCGCCAA	-		GCANCARTET	TCATTCACC	OCCUTCACAA	TACTGAGTAC	CANTACCOTC (OTOMOGENATE .	AACAGAATGA
16901	GICATOCCAT	_		ACTOUTCAGE	ACTUMCCAA	GICATICTOA	GAATAGTOTA TOCOCCOACC		GARTIGCTOT	TOCCOMOCOT
))				Man M. C. Bernye, A.	and Published	CACTABLEACT	CTTATACAT	ACCCCCTOC (CTCNACCAGA	ACCCCCCCCA

Figure 15W

PMRKAd5gag MER682

	ID NO: 27)	taat (seq Vita (seq	FEMILE TO CENTRALE TO A COCCETANGA P	GATCH CATCHER ACCTATANA ATAGGCGTAT GACGACGCC TTTCGTCTTC AAGAATTGA TCGAATTCT TAAT (SEQ ID NO: 27) GTACTATAAT TGGATATTTT TATCCGCATA GTGCTCCTGG AAAGAATAA TTCTTAACT AGGCTTAAA ATTA (SEQ ID NO: 28)	PTICGICTIC AAAGGAGAAG	CACGAGGCCC	ATACECETAT TATCCCCATA	ACCTATAMA FGGATATTTT	CATGACATTA	37401
CCTANTANTA	CAGATICTIT	COCTODACTO	GGGCTTTTCA	GCCTGTANG	TECECONARE	TATTIGITIA	TAMATCTITT	TAAACTTACA	COCCTATOTA	
CCATTATTA	GTCTAAGAAA	CCACCTOAC	CCCGANAGE O	CCKACATTRE	ACCIPATITION	ATAMCAMT	ATTTAGAMAA	ATTICAATCT	CCCCATACAT	37301
COCCUPING ACCUTATION CONCINUES TATACAMENT ATTACAMENTA GAMERAMAN CITATAMENTA CATCOTAMAT ACTOCCAMEN ACAGACTACT	AGTOCCAATA	TTCOTAAAT	GTTATATAA C	CAACTIAAAAA	ATTEACTATE	TTACACTT	CCCCTRITCIC	TCCCTTATTC	CCCCOTTTT	10916
TICCCITITION	grintence	CACCCACTC	CTYXCTCCAA A	CANATEMAN (AGAACTICITA	Traditionit	COTCACCACG	AGCTACATTO	CTCTAGOTCA	
MUCCUMANT	CARNARCACO	CTCCCTCAG	CACCAGGGT 1	CTTTTACTTT (TY:TTY:ACKTAT	ACTEANETES	CCACTCGTGC	TCGATGTAAC	DADATECAGT	37101
ATCCCCACA	AGTICCTAGA	CCTTTTCAG	באעניאטניכנ נ	TAACCITTING (TY: ACCARTAN	CTINCAANTIT	CCTOTATEGE	ATTATOCCOC	OPTUTORCCCT	
בערו ויר וויר ו	יייושאאייי	COMMENT	STREET, CORRECT CO	ATTRICTION OF C	ACTION TO A TO		CCACATARCA	TATACTOCO	CANCACCODA	37001

Figure 15X



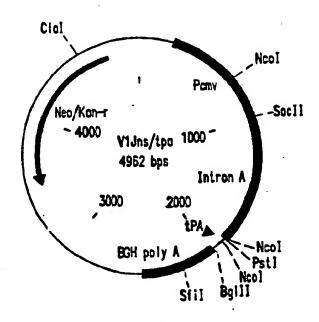


FIGURE 16

GCAGTGGCCCCTGACTGAGGAGAAGATCAAGGCCCTGCTGGAAATCTGCACTGAGATGGAGAAGGAGGGCAAAATCTCCA sGInTrpProLeuThrGluGluLyslleLysAloLeuVolGlulleCysThrGluMelGluLysGluGlyLyslleSerL 30 40 50

AGATTGGCCCGGAGAACCCCTACAACACCCCTGTGTTTGCCATCAAGAAGAAGAAGGACTCCACCAAGTGGAGGAACCTGGTG
yslieGlyProGluAsnProTyrAsnThrProVolPheAlolleLysLysLysAspSerThrLysTrpArgLysLeuVol
60 70

GACTTCAGGGAGCTGAACAAGAGGACCCCAGGACTTCTGGGAGGTGCAGCTGGGCATCCCCCACCCCGCTGGCCTGAAGAA AspPheArgGTuLeuAsnLysArgThrGTnAspPheTrpGTuVoTGTnLeuGTyTleProHisProAloGTyLeuLysLy 80 90 100

GAAGAAGTCTGTGACTGTGGCTGTGGGCGATGCCTACTTCTCTGTGCCCCTGGATGAGGACTTCAGGAAGTACACTG slyslysSerVolThrVolLeu<u>Alo</u>VolGlyAspAloTyrPheSerVolProLeuAspG1uAspPheArgLysTyrThrA 110 120 130

CCTTCACCATCCCCTCCATCAACAATGASACCCCTGGCATCAGGTACCAGTACAATGTGCTGCCCCAGGGCTGSAAGGGC loPheTnrlleProSerlleAsnAsnGluThrProGlylleArgTyrGlnTyrAsnVolLeuProGlnGlyTrpLysGly 140 150

TCCCCTGCCATCTTCCAGTCCTCCATGACCAAGATCCTGGAGCCCTTCAGGAAGCAGAACCCTGACATTGTGATCTACCA SerProAlollePheGInSerSerMetThrLyslleLeuGluProPheArgLysGInAsnProAsplleVollleTyrG1

TGCTGAGGTGGGGCTGACCACCCTGACAAGAAGCACCAGAAGGAGCCCCCCTTCCTGTGGATGGGCTATGAGCTGCAC euleuArgTrpGTyLeuThrThrProAsplysLysHisGInLysGTuProProPheLeuTrpMetGTyTyrGTuLeuHis 220 230

CCCGACAGTGGACTGTGCACCCATTGTGCTGCCTGAGAAGGACTCCTGGACTGTGAATGACATCCAGAAGCTGGTGGG ProAsplystrpThrVoIGinProIieVoileuProGlulysAspSerTrpThrVoiAsnAspIieGinLysLeuVoiGI 240 250 260

CAAGCTGAACTGGGCCTCCCAAATCTACCCTGGCATCAAGGTGAGGCAGCTGTGCAAGCTGCTGAGGGGCACCAAGGCCC yLysLeuAsnTrpAioSerGinlieTyrProGiylieLysVoiArgGinLeuCysLysLeuLeuArgGiyThrLysAioL 270 280 290

FIGURE 17A

TGACTGAGGTGATCCCCCTGACTGAGGAGGCTGAGCTGGAGCTGGGCTGAGAACAGGGAGATCCTGAAGGAGCCTGTGCAT EUThrGIuVollieProLeuThrGIuGIuAIoGIuLeuGiuLeuAIoGIuAsnArgCIulieLeuLysGIuProVolHis 300 310

GCCCTGTACTATGACCCCTCCAACGACCTGATTGCTGAGATCCAGAAGCAGGGCCAGGGCCCAGTGGACCTACCAAATCTA GiyVoiTyrTyrAspProSerLysAspLeuiteAtoGtuiteGinLysGtnGtyGtnGtyGtnTrpThrTyrGtn1teTy 320 340

CCADGAGCCCTTCAAGAACCTGAAGACTGCCAAGTATCCCAGGATGAGGGGGGCCCCACACCAATGATGTGAAGCAGCTGA rGinGluProPheLysAsnLeuLysThrGlyLysTyrAloArgMeLArgGlyAloHisThrAsnAspVolLysGInLeuT 350 370

C1GAGGCTGTGCAGAAGATCACCACTGAGTCCATTGTGATCTGGGGCAAGACCCCCAAGTTCAAGCTGCCCATCCAGAAG hrGluAloVolGinLyslleThrThrGluSerlleVollleTrpGlyLysThrProLysPheLysLeuProlleGinLys 380 390

GGTGAAGCTGTGGTACCAGCTGGAGAAGGAGCCCATTGTGGGGGGCTGAGACCTTCTATGTGGCTGGGGCTGCCAACAGGG uVollysleu1rpTyrGinleuGiuLysGiuProlleVolGlyA1gGiuThrPheTyrVolAlgGyA1gA1gAsnArgG 430 440 450

AAGACTGCCCTCCAGGCCATCTACCTGGCCCTCCAGGACTCTGGCCTGGAGGTGAACATTGTGACTGCCTCCCAGTATGC
LysThrAloLeuGinAlolleTyrLeuAloLeuGinAspSerGlyLeuGluVolAsnIleVolThrAloSerGInTyrAl
480
490
500

CCTGGGCATCATCCAGGCCCAGCCTGATCAGTCTGAGCTCGAGCTGGTGAACCAGATCATTGAGCAGCTGATCAAGAAGG oLeuGiylielieGinAioGinProAspGinSerGiuSerGiuLeuVoiAsnGinIielieGiuGinLeuIieLysLysG 510 520 530

AGAAGGTGTACCTGCCCTGCCCGCCACAAGGCCATTGCGGGCAATGAGCAGGTGGACAAGCTGGTGTCTGCTGGC
IULysVolTyrLeuAloTrpVolProAloHisLysGiylleGiyGiyAsnGluGinVolAspLysLeuVolSerAloGiy
540
550

ATCAGGAAGGTGCTGTTCCTGGATGGCATTGACAACCCCCAGGATGAGCATGAGAAGTACCACTCCAACTGGAGGGCTAT
11eArgLysVolleuPheleuAspG1y11eAspLysA1oG1nAspG1uHisG1uLysTyrHisSerAsnTrpArgA1oMe
560 570 580

FIGURE 17B

GCCCTCTGACTTCAACCTGCCCCCTGTGGTGGCTAAGGAGATTGTGGCCTCCTGTGACAAGTGCCAGCTGAAGGCCGAGG tAloSerAspPheAsnLeuProProVolVolAloLysGlulleVolAloSerCysAspLysCysGlnLeuLysGlyGluA 590 600 610

CCATGCATGGGCAGGTGGACTGCTCCCCTGGCATCTGGCAGGTGACCCACCTGGAGGGCAAGGTGATCCTGGTG IdMetHisGlyGlnVolAspCysSerProGlyIteTrpGlnLeuAloCysThrHisLeuGluGlyLysVolIteLeuVol 620 630

GCTGTGCATGTGGCCTCCGGCTACATTGAGGCTGAGGTGATCCCTGCTGAGACAGGCCAGGAGACTGCCTACTTCCTGCT AlovolHisVolAloSerGlyTyrlleGluAloGluVollleProAloGluThrGlyGlnGluThrAloTyrPheLeuLe 640 650 660

GAAGCTGGCTGGCAGGTGGCCTGTGAAGACCATCCACACTGCCAATGGCTCCAACTTCACTGGGCCCACAGTGAGGGCTG
uLysLeuAldGlyArgTrpProVolLysThrIleHisThrAloAsnGlySerAsnPheThrGlyAldThrValArgAloA
680
690

CCTGCTGGTGGCCTGCCATCAASCAGGAGTTTGGCATCCCCTACAACCCCCAGTCCCACGGGTGGTGGCCTCCATGAAC
IOCysTrpTrpAIoGlyIleLysGInGluPheGlyIleProTyrAsnProGInSerGInGlyVolVolAloSerMelAsn
700 710

AAGGAGCTGAAGAAGATCATTGGGCAGGTGAGGGACCAGCTGAGCACCTGAAGACAGCTGTGCAGATGGCTGTGTTCAT LysGluLeuLysLysllelleGlyGlnVolArgAspGlnAloGluHisLeuLysThrAloVolGlnMetAloVolPhell 720 730 740

CCACAACTTCAAGAGGAAGGGGGCATCGGGGGCTACTCGGCTGGGGAGAGGATIGTGGACATCATTGCCACAGACATCC
eHisAsnPhelysArgLysGlyGlylleGlyGlyTyrSerAloGlyGluArglleVolAsplleIleAloThrAsplleG
750
760
770

AGACCAAGGAGCTCCAGAAGCAGATCACCAAGATCCAGAACTTCAGGGTGTACTACAGGGACTCCAGGAACCCCCTGTGG
InThrLysGIuLeuGInLysGInlieThrLysIieGInAsnPheArgVoITyrTyrArgAspSerArgAsnProLeuTrp
78D 79D

AAGGCCCTGCCAAGCTGCTGTGGAAGCGGGAGCGGGTGCTGGTGATCCAGGACACTCTGACATCAAGGTGGTGCCCAG LysGtyProAtolysLeuleuTrpLysGtyGtuGtyAtoVotVotIteGtnAspAsnSerAsp1teLysVotVotProAr 800 810 820

AAAGCCCCCCCAGATCT (SEQ ID NO: 3)
Xx Ball (SEQ ID NO: 4)

FIGURE 17C

(within SEQ 10 NO: 7) (within SEQ 10 NO: 8) CCACCCAGAGATCTCCCCCCATCTCCCCCATTGAGACTGTGCCTGTGAAGCTGAAGCTGCCATGGCTGCC RoSarGiulieSerAidProlleSerProlleGluThrVolProValLysLeuLysProGlyMelAspGly 10 20 20

FIGURE 18

ਮਾ		-42
OPT	- ÁTG GÁC GÁC ÁÁG TÁG TÉC ÁÁG AĞG TCC ĞTĞ ČÉC ĞĞC TĞĞ TÉC M G G K W S K R S V P G W S	-14
WT	- ACT GTA AGG GAA AGA ATG AGA CGA GCT GAG CCA GCA GAT	-84
OPT	- ÁCC GTG ÁGG GÁG ÁGG ÁTG ÁGG AGG GCC GÁG CCC GCC GÁC T V R E R H R R A E P A A D	-28
WT.	- AGG GTG AGA CGA ACT GAG CCA GCA GCA GTA GGG GTG GGA GCA	-126
OPT	- ÁĞĞ ĞTĞ ÁĞG AĞG ÁCC ĞÁĞ CCC ĞCC ĞCC ĞTĞ ĞĞC ĞTĞ ĞĞC ĞCC R V R R T E P A A V G V G A	-42
WT	- GTA TCT CGA GAC CTG GAA AAA CAT GGA GCA ATC ACA AGT AGC	-168
OPT	- GTG TCC AGG GÁC CTG GÁG ÁÁG CÁC GGC GCC ÁTC ÁCC TCC TCC V S R D L E K H G A I T S S	-56
WT	- AAT ACA GCA GCT ACC AAT GCT GAT TGT GCC TGG CTA GAA GCA	-210
OPT	- ÁÁC ÁCC GCC ÁCC ÁÁC GCC GÁC TGC GCC TGG CTG GÁG GCC N T A A T N A D C A W L E A	-7 0.
WT	- CAA GAG GAT GAG GAA GTG GGT TTT CCA GTC AGA CCT CAG GTA	-252
OPT	- CÁG GÁG GÁC GÁG GÁG GTG GGC TTC CCC GTG ÁGG CCC CÁG GTG Q E D E E V G F P V R P Q V	-84
WT	- CCT TTA AGA CCA ATG ACT TAC AAG GGA GCT GTA GAT CTT AGC	-294
OPT	- CCC CTG AGG CCC ATG ACC TAC AAG GGC GCC GTG GAC CTG TCC P L R P M T Y K G A V D L S	-98
WT	- CAC TIT TTA AAA GAA AAG GGG GGA CTG GAA GGG CTA ATT CAC	-336
OPT	- CÁC TÍC CTG ÁAG GÁG ÁAG GÉC ÉTG ÉAG GÉC ÉTG ÁTC CÁC H F L K E K G G L E G L I H	-112
WT	- TCA CAG AAA AGA CAA GAT ATC CTT GAT CTG TGG GTC TAC CAC	-378
OPT	- TCC CAG AAG AGG CAG GAC ATC CTG GAC CTG TGG GTG TAC CAC S Q K R Q D I L D L W V Y H	-126
WT	- ACA CAA GGC TAC TTC CCT GAT TGG CAG AAC TAC ACA CCA GGG	-420
OPT	- ACC CAG GGC TAC TTC CCC GAC TGG CAG AAC TAC ACC CCC GGC T Q G Y F P D W Q N Y T P G	-140

FIGURE 19A

WT	- CCA GGA ATC AGA TIT CCA TTG ACC TTT GGA TGG TGC TTC AAG -462	
OPT	- CCC GGC ATC AGG TTC CCC CTG ACC TTC GGC TGG TGC TTC AAG P G I R F P L T F G W C F K -154	
WT	- CTA GTA CCA GTT GAG CCA GAA AAG GTA GAA GAG GCC AAT GAA -504	
OPT	- CTG GTG CCC GTG GÁG CCC GÁG ÁÁG GTG GÁG GCC ÁÁC GÁG L V P V E P E K V E E A N E -168	
WT	- GGA GAG AAC AAC TGC TTG TTA CAC CCT ATG AGC CAG CAT GGG -546	
OPT	- GÁC GÁG ÁÁC ÁÁC TỚC CTỔ CTĆ CÁC CÓC ÁTỔ TCĆ CÁG CÁC GÁC G E N N C L L H P M S Q H G -182	
WT	- ATA GAG GAC CCG GAG AAG GAA GTG TTA GAG TGG AGG TTT GAC -588	i
OPT	- ATC GAG GAC CCC GAG AAG GAG GTG CTG GAG TGG AGG TTC GAC 1 E D P E K E V L E W R F D -196	,
WT .	- AGC AAG CTA GCA TTT CAT CAC GTG GCC CGA GAG CTG CAT CCG -630)
OPT	- TCC AAG CTG GCC TTC CAC CAC GTG GCC AGG GAG CTG CAC CCC S K L A F H H V A R E L H P -210)
WT	- GAG TAC TAC AAG GAC TGC TGA (SEQ ID NO:30) -651	l
OPT	- GAG TAC TAC AAG GAC TGC TAA (contained within SEQ ID NO: 9) E Y Y K D C (SEQ ID NO: 10) -216	5

FIGURE 19B

VIJns/nef

CARGASTICTITIC LIGITALISTICS CALCE ATG GGC GGC ANG TGG TCC ANG NGG TCC GTG CCC .

M G G K W S K R S V P

Srf1 Ball1 CAC CCC GAG TAC TAC AAG GAC TGC TAA AGCCCGGGAACAGAICTGCCTTCTAGTTGCCAGC (SEQ 1D NU: 38) H P E Y Y K D C * (contained within SEQ 1D NO: 10:

V1Jns/nef(G2A.LLAA)

Psti CATBASTETTTICIBICALCESTECTTBA<u>GAITCI</u>BACCACC ATG GCC GGC AAG TGG TCC AAG AGG TCC GTG CCC . M A G K W S K R S V P

Srff Balls

CAC CCC GAG TAC TAC AAG GAC TGC TAA AGCCCGGGCACAICTGCTGTGCCTTCTAGTTGCCAGC (SEQ 10 NO: 39)

H P E Y Y K D C * (contained within SEQ 1D NO:14)

VlJns/tpanef & VlJns/tpanef(LLAA)

Pst1 Catgggicticaegicaecegicaecettaiaictaeaicaec atg gat gca atg ang aga ctc tgc tgt gtg m d a m k r g l c c v

CTG CTG CTG TGT GGA GCA GTC TTC GTT TCG CCC AGC GAG AIC ICC TCC AAG AGG TCC GTG CCC ...

FIGURE 20

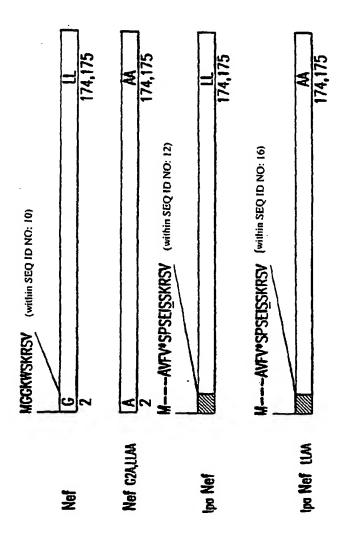


FIGURE 21

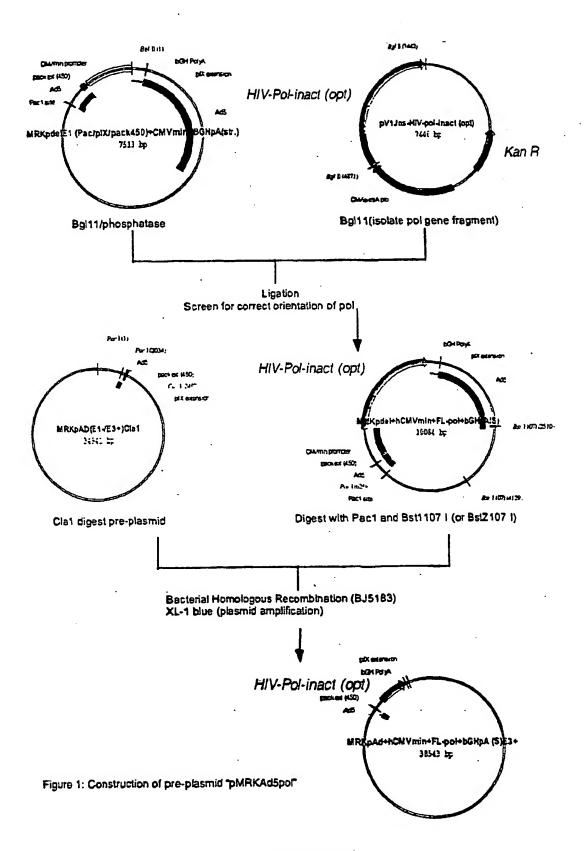


FIGURE 22

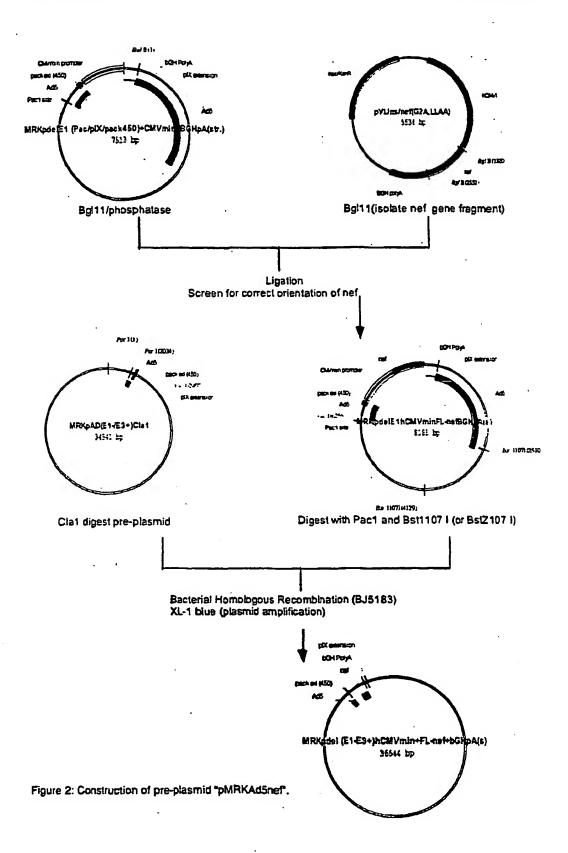


FIGURE 23

Comparison of Clade B vs. Clade C Anti-gag T Cell Responses in Clade B HIV-Infected Subjects

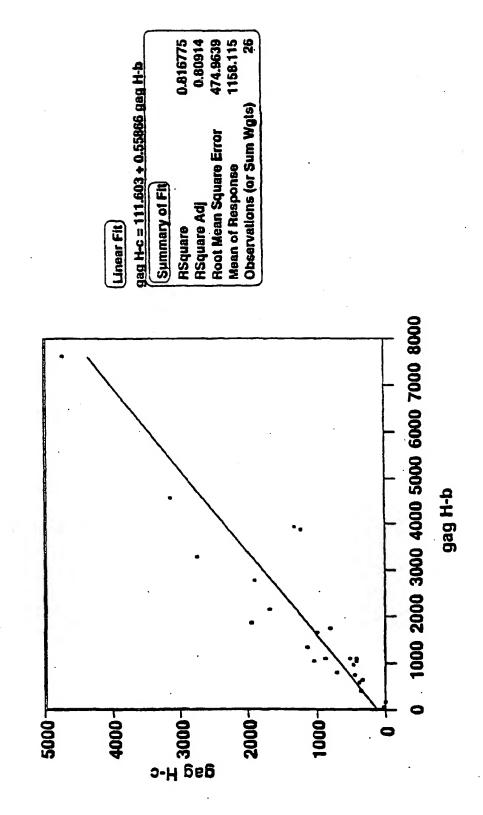
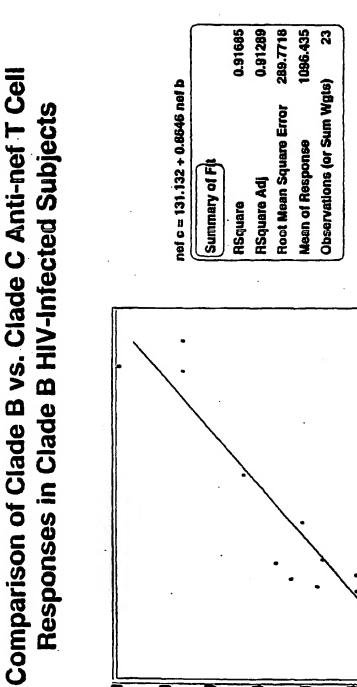


FIGURE 25



2500

2000

1500

o ten

1000

3500

3000

MRKAd5pol MER1062 (MRKAd5 Pre-Adenoviral Vector Containing the IA opt pol Coding Region)

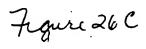
1	CATCATCAAT	AATATACCTT	ATTTTGGATT	GAAGCCAATA	TGATAATGAG
	GTAGTAGTTA	TTATATGGAA	TAAAACCTAA	CTTCGGTTAT	OTOKTTATOA
51	GGGGTGGAGT	TTGTGACGTG	GCGCGGGGCG	TGGGAACGGG	GCGGGTGACG
	CCCCACCTCA	AACACTGCAC	CGCGCCCCGC	ACCCTTGCCC	CGCCCACTGC
101	TAGTAGTGTG	GCGGAAGTGT	GATGTTGCAA	GTGTGGCGGA	ACACATGTAA
			CTACAACGTT		
151	GCGACGGATG	TGGCAAAAGT	GACGTTTTTG	GTGTGCGCCG	GTGTACACAG
			CTGCAAAAAC		
			GTTTTAGGCG		
			CAAAATCCGC		
251	CGTAACCGAG	TAAGATTTGG	CCATTTTCGC	GGGAAAACTG	AATAAGAGGA
			GGTAAAAGCG		
301	AGTGAAATCT	GAATAATTT	GTGTTACTCA	TAGCGCGTAA	TATTIGICIA
			CACAATGAGT		
351	GGGCCGCGG	GACTITGACC	GTTTACGTGG	AGACTCGCCC	AGGTGTTTTT
			CAAATGCACC		
401			CGGGTCAAAG		
			GCCCAGTTTC		
451	GCGGCCGCGA	TCCATTGCAT	ACGITGTATC	CATATCATAA	TATGTACATT
				•	ATACATGTAA
501					GATTATTGAC
					CTAATAACTG
551					AGCCCATATA
					TCGCGTATAT
601					TGGCTGACCG
					ACCGACTGGC
651	CCCAACGACC	CCCGCCCATI	GALGICAAIA	ATGACGTATG	TTCCCATAGT AAGGGTATCA
701	AACGCCAATA	GGGACTITCC	ATIGACGICA	ATGGGTGGAG	TATTTACGGT
					ATAAATGCCA
751	AAACTGCCCA	CTTGGCAGTA	CATCAAGIGT	ATCATATGCC	AAGTACGCCC
					TTCATGCGGG
801	CCTATTGACG	TCAATGACGG	TAAATGGCCC	GCCTGGCATT	ATGCCCAGTA
					TACGGGTCAT
851	CATGACCTTA	TEGGACTITY	CTACTTGGCA	GTACATCTAC	GTATTAGTCA CATAATCAGT
	CTACTGGAAT	· ACTTTGAAAC	GATGAACCGT	CATGTAGATO	CATAATCAGT

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O 02/02.	2080				PC17
901				AGTACATCAA TCATGTAGTT	
951				CTCCACCCCA GAGGTGGGGT	
1001				GGACTTTCCA CCTGAAAGGT	
1051				GTAGGCGTGT CATCCGCACA	
1101				CGTCAGATCG GCAGTCTAGC	
1151				ACACCGGGAC TGTGGCCCTG	
1201	TCCGCGGCCG	GGAACGGTGC	ATTGGAACGC	GGATTCCCCG CCTAAGGGGC	TGCCAAGAGT
1251	GAGATCTACC	ATGGCCCCCA	TCTCCCCCAT	TGAGACTGTG ACTCTGACAC	CCTGTGAAGC
1301	TGAAGCCTGG	CATGGATGGC	CCCAAGGTGA	AGCAGTGGCC TCGTCACCGG	CCTGACTGAG
1351	GAGAAGATCA	AGGCCCTGGT	GGAAATCTGC	ACTGAGATGG	AGAAGGAGGG
1401	CAAAATCTCC	AAGATTGGCC	CCGAGAACCC	TGACTCTACC CTACAACACC	CCTGTGTTTG
1451				GATGTTGTGG GGAAGCTGGT	
1501				CCTTCGACCA GAGGTGCAGC	
1551	CTCGACTTGT	TCTCCTGGGT	CCTGAAGACC	CTCCACGTCG	ACCCGTAGGG
	GGTGGGGCGA	CCGGACTTCT	TCTTCTTCAG	ACACTGACAC	GACCGACACC
		GAAGAGACAC	GGGGACCTAC	TCCTGAAGTC	CTTCATGTGA
		AGGGGAGGTA	GTTGTTACTC	TGGGGACCGT	AGTCCATGGT
1701	GTACAATGTG CATGTTACAC			CTCCCTGCC GAGGGGACGG	
1751	CCTCCATGAC GGAGGTACTG				
1801	GTGATCTACC CACTAGATGG	•			



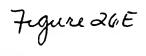
1901	GGGGCCTGAC CCCCGGACTG	CACCCCTGAC GTGGGGACTG		
1951		ATGAGCTGCA TACTCGACGT		
2001	•••••	AAGGACTCCT TTCCTGAGGA		
2051		CTGGGCCTCC GACCCGGAGG		
2101		TGCTGAGGGG ACGACTCCCC		
2151	GACTGAGGAG CTGACTCCTC	GCTGAGCTGG CGACTCGACC		
2201		TGGGGTGTAC ACCCCACATG		
2251		AGGGCCAGGG TCCCGGTCCC		
2301		CTGAAGACTG GACTTCTGAC		
2351		GAAGCAGCTG CTTCGTCGAC		
2401		TCTGGGGCAA AGACCCCGTT		
2451		GAGACCTGGT CTCTGGACCA		
2501		GTTTGTGAAC CAAACACTTG		
2551		AGCCCATTGT TCGGGTAACA		
2601	TGCCAACAGG ACGGTTGTCC	GAGACCAAGC CTCTGGTTCG		
2651	GCAGGCAGAA CGTCCGTCTT			GAAGACTGCC CTTCTGACGG
2701				AGGTGAACAT TCCACTTGTA
2751				CAGCCTGATC GTCGGACTAG



2851	GAGAAGGTGT CTCTTCCACA			CACAAGGGCA GTGTTCCCGT	
2901				CATCAGGAAG GTAGTCCTTC	= -
2951				ATGAGAAGTA TACTCTTCAT	
3001		· · · · · · · · · · · · · · · · · · ·		CCCCCTGTGG GGGGGACACC	
3051	CTAACACCGG	AGGACACTGT	TCACGGTCGA	GAAGGGGGAG CTTCCCCCTC	CGGTACGTAC
3101	CCGTCCACCT	GACGAGGGGA	CCGTAGACCG	AGCTGGCCTG TCGACCGGAC	GTGGGTGGAC
3151	CTCCCGTTCC	ACTAGGACCA	CCGACACGTA	GTGGCCTCCG CACCGGAGGC	CGATGTAACT
3201	CCGACTCCAC	TAGGGACGAC	TCTGTCCGGT	GGAGACTGCC CCTCTGACGG	ATGAAGGACG
3251	ACTTCGACCG	ACCGTCCACC	GGACACTTCT	CCATCCACAC GGTAGGTGTG	ACGGTTACCG
3301	AGGTTGAAGT	GACCCCGGTG	TCACTCCCGA		CCCGACCGTA
3351	GTTCGTCCTC	AAACCGTAGG	GGATGTTGGG	CCAGTCCCAG GGTCAGGGTC	CCCCACCACC
3401		GTTCCTCGAC	TTCTTCTAGT	AACCCGTCCA	CTCCCTGGTC
	CGACTCGTGG	ACTTCTGTCG	ACACGTCTAC	•	AGGTGTTGAA
3501	GTTCTCCTTC	CCCCCGTAGC	CCCCGATGAG	CGCTGGGGAG GCGACCCCTC	TCCTAACACC
		GTGTCTGTAG	GTCTGGTTCC	TCGAGGTCTT	CGTCTAGTGG
	•	TGAAGTCCCA	CATGATGTCC	CTGAGGTCCT	TGGGGGACAC
		CGGTTCGACG	ACACCTTCCC	CCTCCCCGA	CACCACTAGG
3701	AGGACAACTC TCCTGTTGAG				CAAGATCATC GTTCTAGTAG

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3801	GGATGAGGAC CCTACTCCTG		GCAGATCTGC CGTCTAGACG		
3851			CCCGTGCCTT GGGCACGGAA		
3901.	ACTCCCACTG TGAGGGTGAC		ATAAAATGAG TATTTTACTC		
3951			TGGGGGGTGG ACCCCCCACC		
4001			AGCAGGCATG TCGTCCGTAC		
4051			ACTGAAATGT TGACTTTACA		
4101	CCTTTCTTAT	ATATTCCACC	GGGTCTTATG CCCAGAATAC	ATCAAAACAT	AGACAAAACG
4151	TCGTCGGCGG	CGGCGGTACT	GCACCAACTC CGTGGTTGAG	CAAACTACCT	TCGTAACACT
4201	CGAGTATAAA	CTGTTGCGCG	ATGCCCCCAT TACGGGGGTA	CCCGGCCCCA	CGCAGTCTTA
4251	CACTACCCGA	GGTCGTAACT	TGGTCGCCCC ACCAGCGGGG	CAGGACGGGC	GTTTGAGATG
4301	ATGGAACTGG	ATGCTCTGGC	TGTCTGGAAC ACAGACCTTG	CGGCAACCTC	TGACGTCGGA
4351	GGCGGCGGCG	AAGTCGGCGA	GCAGCCACCG CGTCGGTGGC	GGGCGCCCTA	ACACTGACTG
4401			TGCAAACAGT ACGTTTGTCA		
4451	GGCGCTACTG	TTCAACTGCC		TGTTAACCTA	AGAAACTGGG
	CCCTTGAATT	ACAGCAAAGA	GTCGTCGACA	ACCTAGACGC	CCAGCAGGTT GGTCGTCCAA
	AGACGGGACT	TCCGAAGGAG	GGGAGGGTTA	CGCCAAATTT	ACATAAATAA TGTATTTATT
4601	AAAACCAGAC TTTTGGTCTG	TCTGTTTGGA AGACAAACCT	TTTGGATCAA AAACCTAGTT	GCAAGTGTCT CGTTCACAGA	TGCTGTCTTT ACGACAGAAA
4651	ATTTAGGGGT TAAATCCCCA	TTTGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGC	CGGTAGGCCC GCCATCCGGG	GGGACCAGCG CCCTGGTCGC	GTCTCGGTCG CAGAGCCAGC



4751	GTTCAGATAC	ATGGGCATAA	GCCCGTCTCT	GGGGTGGAGG	TAGCACCACT
				CCCCACCTCC	
4801	GCAGAGCTTC	ATGCTGCGGG	GTGGTGTTGT	AGATGATCCA	GTCGTAGCAG
				TCTACTAGGT	
4851				TTCAGTAGCA	
				AAGTCATCGT	
4901	•••••			AAAGCGGTTA TTTCGCCAAT	
4951				TGGACTGTAT ACCTGACATA	
5001				TTCATGTTGT AAGTACAACA	
	CGATACAAGG	GICGGIAIAG	GGAGGCCCCI	ANGIACAACA	CG1C11GG1G
5051				TTTGTCATGT	
	GTCGTGTCAC	ATAGGCCACG	TGAACCCTTT	AAACAGTACA	TCGAATCTTC
5101	CAAATCCCTC	CAACAACTTC	CACACCCCCT	TGTGACCTCC	ልልርልጥጥጥጥርር
5101				ACACTGGAGG	
5151				CCACGGGCGG	
				GGTGCCCGCC	
5201				GTTGTGTTCC	
				CAACACAAGG	
5251				GGAGGGTGCC	
	•			CCTCCCACGG	
-5301				TTACCCTCAC	
5351				CATGTCTACC	
	AAGGGTGCGA	AACTCAAGTC	TACCCCCCTA	GTACAGATGG	ACGCCCCGCT
5401	TGAAGAAAAC	GGTTTCCGGG	GTAGGGGAGA	TCAGCTGGGA	AGAAAGCAGG
				AGTCGACCCT	
5451	TTCCTGAGCA	GCTGCGACTT	ACCGCAGCCG	CTGGGCCCGT	AAATCACACC
					TTTAGTGTGG
5501	TATTACCGGC				
	ATAATGGCCG	ACGTTGACCA	TCAATTCTCT	CGACGTCGAC	GGCAGTAGGG
5551					
	ACTCGTCCCC	CCGGTGAAGC	AATTCGTACA	GGGACTGAGC	GTACAAAAGG
5601	CTGACCAAAT				
	GACTGGTTTA	GGCGGTCTTC	CGCGAGCGGC	GGGTCGCTAT	CGTCAAGAAC



5701		TTGACCAAGC AACTGGTTCG			
5751		CATCTCGATC GTAGAGCTAG			
5801	CGGCTTTCGC	TGTACGGCAG	TAGTCGGTGC	TCGTCCAGAC	GGGCCAGGGT
	GCCGAAAGCG	ACATGCCGTC	ATCAGCCACG	AGCAGGTCTG	CCCGGTCCCA
5851		CACGGGCGCA GTGCCCGCGT			
5901		CGCTCCGGGC GCGAGGCCCG			
5951	GTCCTGCTGG	TGCTGAAGCG	CTGCCGGTCT	TCGCCCTGCG	CGTCGGCCAG
	CAGGACGACC	ACGACTTCGC	GACGGCCAGA	AGCGGGACGC	GCAGCCGGTC
6001	GTAGCATTTG	ACCATGGTGT	CATAGTCCAG	CCCCTCCGCG	GCGTGGCCCT
	CATCGTAAAC	TGGTACCACA	GTATCAGGTC	GGGGAGGCGC	CGCACCGGGA
6051		CTTGCCCTTG GAACGGGAAC			
6101		CGTAGAGCTT GCATCTCGAA			
6151		CCGCAGGCCC GGCGTCCGGG			
6201	TGAGCTCTGG	CCGTTCGGGG	TCAAAAACCA	GGTTTCCCCC	ATGCTTTTTG
	ACTCGAGACC	GGCAAGCCCC	AGTTTTTGGT	CCAAAGGGGG	TACGAAAAAC
6251	ATGCGTTTCT	TACCTCTGGT	TTCCATGAGC	CGGTGTCCAC	GCTCGGTGAC
	TACGCAAAGA	ATGGAGACCA	AAGGTACTCG	GCCACAGGTG	CGAGCCACTG
6301	GAAAAGGCTG	TCCGTGTCCC	CGTATACAGA	CTTGAGAGGC	CTGTCCTCGA
	CTTTTCCGAC	AGGCACAGGG	GCATATGTCT	GAACTCTCCG	GACAGGAGCT
6351	GCGGTGTTCC	GCGGTCCTCC	TCGTATAGAA	ACTCGGACCA	CTCTGAGACA
	CGCCACAAGG	CGCCAGGAGG	AGCATATCTT	TGAGCCTGGT	GAGACTCTGT
6401	AAGGCTCGCG	TCCAGGCCAG	CACGAAGGAG	GCTAAGTGGG	AGGGGTAGCG
	TTCCGAGCGC	AGGTCCGGTC	GTGCTTCCTC	CGATTCACCC	TCCCCATCGC
6451	GTCGTTGTCC	ACTAGGGGGT	CCACTCGCTC	CAGGGTGTGA	AGACACATGT
	CAGCAACAGG	TGATCCCCCA	GGTGAGCGAG	GTCCCACACT	TCTGTGTACA
6501	CGCCCTCTTC	GGCATCAAGG	AAGGTGATTG	GTTTGTAGGT	GTAGGCCACG
	GCGGGAGAAG	CCGTAGTTCC	TTCCACTAAC	CAAACATCCA	CATCCGGTGC
6551	TGACCGGGTG	TTCCTGAAGG	GGGGCTATAA	AAGGGGGTGG	GGGCGCGTTC
	ACTGGCCCAC	AAGGACTTCC	CCCCGATATT	TTCCCCCACC	CCCGCGCAAG

Figure 266

6651	AGTACTCCCT TCATGAGGGA		GGCATGACTT CCGTACTGAA		
6701			GATATTCACC CTATAAGTGG		
6751			GGTCAGAAAA CCAGTCTTTT		
6801			TAGAGGGCGT ATCTCCCGCA		
6851			GTCGCGATCG CAGCGCTAGC		
6901	CAAATCGACG	TGCATAAGCG	GCGCAACGCA CGCGTTGCGT	GGCGGTAAGC	CCTTTCTGCC
6951	ACCACGCGAG	CAGCCCGTGG	AGGTGCACGC TCCACGTGCG	CGGTTGGCGC	CAACACGTCC
7001	CACTGTTCCA	GTTGCGACCA	GGCTACCTCT CCGATGGAGA	GGCGCATCCG	CGAGCAACCA
7051	GGTCGTCTCC	GCCGGCGGGA	TGCGCGAGCA ACGCGCTCGT	CTTACCGCCA	TCCCCCAGAT
7101	CGACGCAGAG	CAGGCCCCCC	TCTGCGTCCA AGACGCAGGT	GCCATTTCTG	GGGCCCGTCG
7151	TCCGCGCGCA	GCTTCATCAG	TATCTTGCAT ATAGAACGTA	GGAACGTTCA	GATCGCGGAC
7201	GACGGTACGC	GCCCGCCGTT	GCGCGCGCTC CGCGCGCGAG	CATACCCAAC	TCACCCCCTG
7251	GGGTACCGTA	CCCCACCCAC	AGCGCGGAGG TCGCGCCTCC	GCATGTACGG	CGTTTACAGC
7301	ATTTGCATCT	CCCCGAGAGA	CTCATAAGGT	TCTATACATC	GGTAGCATCT CCATCGTAGA
	AGGTGGCGCC	TACGACCGCG	CGTGCATTAG	CATATCAAGC	TGCGAGGGAG ACGCTCCCTC
	GCTCCTCCAG	CCCTGGCTCC	AACGATGCCC	GCCCGACGAG	TGCTCGGAAG ACGAGCCTTC
	TGATAGACGG	ACTTCTACCG	TACACTCAAC	CTACTATACC	TTGGACGCTG AACCTGCGAC
7501					CGCACGAAGG

Figure 26 H

7601			ATGATGTCAT TACTACAGTA	
7651			GACAAACTCT CTGTTTGAGA	
7701			CCTCCGAACG GGAGGCTTGC	
7751			GCGCAGCATC CGCGTCGTAG	
7801		 	GAGCGAGGTG CTCGCTCCAC	
7851	CAAAGGTGTC GTTTCCACAG		ACTGGTATTT TGACCATAAA	
7901		 	AAGTCCGTGC TTCAGGCACG	
7951			GTTGAAGAGT CAACTTCTCA	
8001			AGGGTCCCGG TCCCAGGGCC	
8051			ATCTCGTCAA TAGAGCAGTT	
8101		 	GCGCGGGATG CGCGCCCTAC	
8151		 	GCTCTTCAGG CGAGAAGTCC	
8201			TGAGGGTTGG ACTĊCCAACC	
8251		 • •	TTGCAGGTGG AACGTCCACC	
8301	TCCTAAACTG AGGATTTGAC		CTGGGGTGAT GACCCCACTA	
8351	GTAAGCGGGT CATTCGCCCA		CCAAGGTTCG GGTTCCAAGC	
8401	TCGCGCGGCA AGCGCGCCGT		GCCGAACTTC CGGCTTGAAG	
8451	TGAAGGGCAC ACTTCCCGTG			ATAGGTCTCT TATCCAGAGA

Figure 26I

8551				GGAGTGGCTA CCTCACCGAT	
8601				ACTCGTGCTG TGAGCACGAC	
8651	AAACGTGCGC	AGTACTGGCA TCATGACCGT	GCGGTGCACG	GGCTGTACAT CCGACATGTA	CCTGCACGAG GGACGTGCTC
8701	GTTGACCTGA	CGACCGCGCA	CAAGGAAGCA	GAGTGGGAAT CTCACCCTTA	TTGAGCCCCT
8751	CGCCTGGCGG	GTTTGGCTGG	TGGTCTTCTA	CTTCGGCTGC GAAGCCGACG	TTGTCCTTGA
8801	CCGTCTGGCT	GCTCGAGGGG	AGTTACGGTG	GATCGGACCA	CCACGCCGCG
			•	CTAGCCTGGT	
8851				CGGTCGGAGC GCCAGCCTCG	
8901				GGAGCTCCCG CCTCGAGGGC	
8951				CATAGACGGG GTATCTGCCC	
9001				GGGCTGGTTG CCCGACCAAC	
9051				GCGCGACTAC CGCGCTGATG	
9101				GATGATGCAT CTACTACGTA	
9151				GGCTCCGGAC CCGAGGCCTG	
9201				GGGCAGGAGC CCCGTCCTCG	TGGTGCTGCG ACCACGACGC
9251	CGCGTAGGTT GCGCATCCAA	GCTGGCGAAC CGACCGCTTG	GCGACGACGC CGCTGCTGCG	GGCGGTTGAT CCGCCAACTA	CTCCTGAATC GAGGACTTAG
9301	TGGCGCCTCT ACCGCGGAGA	GCGTGAAGAC CGCACTTCTG	GACGGGCCCG	GTGAGCTTGA CACTCGAACT	ACCTGAAAGA TGGACTTTCT
9351	GAGTTCGACA CTCAAGCTGT	GAATCAATTT CTTAGTTAAA	CGGTGTCGTT GCCACAGCAA	GACGGCGGCC	TGGCGCAAAA ACCGCGTTTT
9401	TCTCCTGCAC AGAGGACGTG	GTCTCCTGAG CAGAGGACTC	TTGTCTTGAT AACAGAACTA	AGGCGATCTC TCCGCTAGAG	GGCCATGAAC CCGGTACTTG

Figure 26. J 64/144

9501		TCGTTGGAAA AGCAACCTTT			
9551		GTTCCAGACG CAAGGTCTGC			
9,601		TGACCACCTG ACTGGTGGAC			
9651		TTTCGCAGGC AAAGCGTCCG			
9701		CACGAAGAAG GTGCTTCTTC			
9751		CCAAGGCCTC GGTTCCGGAG			
9801		AAAAACTGGG TTTTTGACCC			
9851		GATGAGCTCG CTACTCGAGC			
9901		CCTCTTCTTC GGAGAAGAAG			
9951		TCTTCTGGCG AGAAGACCGC			
10001		CGGGAGGCGG GCCCTCCGCC			
10051		TGGTCTCGGT ACCAGAGCCA			
10101		CCGCCCGTCA GGCGGGCAGT			
10151					TTGTTGTGTA AACAACACAT
10201	GGTACTCCGC CCATGAGGCG	CGCCGAGGGA GCGGCTCCCT	CCTGAGCGAG GGACTCGCTC	TCCGCATCGA AGGCGTAGCT	CCGGATCGGA GGCCTAGCCT
10251					GGTAGGCTGA CCATCCGACT
10301					TCTGGCGGAG AGACCGCCTC
10351					GGCGGATGGT CCGCCTACCA

Figure 26 K

10451	CGGCCATGCC GCCGGTACGG		GGCGCAGGTC CCGCGTCCAG	
10501	TCTTGCATGA AGAACGTACT		TCTTCTCCTT AGAAGAGGAA	
10551		 	GGCGGAGTTT CCGCCTCAAA	
10601			CGAAGCCCCT GCTTCGGGGA	•
10651			GCTAATATGG CGATTATACC	
	CTGCGTGAGG . GACGCACTCC	 		
10751		 	CCATAACGGA GGTATTGCCT	
10801			TACCTGAGAC ATGGACTCTG	
10851	•	 	CCGCACCAGG GGCGTGGTCC	· -
10901			AGAGGGGCCA TCTCCCCGGT	
10951			ATAAGGCGAT TATTCCGCTA	
11001		 	GGCGGTGGTG CCGCCACCAC	
11051			GCAGCGGCAA CGTCGCCGTT	
11101			GCGCAATCGT CGCGTTAGĆA	
11151	GACCGTGCAA CTGGCACGTT		CACTCTTCCG GTGAGAAGGC	
11201	GATAAATTCG CTATTTAAGC		ACCGGGGTTC TGGCCCCAAG	
11251	TCCGGCCGTC AGGCCGGCAG		CCGCCCGCGT	
11301	GGTGTGCGAC CCACACGCTG		TCCTTTTGGC AGGAAAACCG	

Figure 26L

11401				AGTGGCTCGC TCACCGAGCG	
11451				GGGACCCCCG CCCTGGGGGC	
11501				TTGCCTCCCC AACGGAGGGG	
11551				GACGAGCCCC CTGCTCGGGG	
11601				GCGCCCCCT CGCGGGGGGA	
11651	-			GGGCACCCTC CCCGTGGGAG	
11701				GACGCGGCAG CTGCGCCGTC	
11751				CTACCTGGAC GATGGACCTG	
11801	CGCTCCCGGA	CCGCGCCGAT	CCTCGCGGGA	CTCCTGAGCG GAGGACTCGC	CGTGGGTTCC
11851				TACGTGCCGC ATGCACGGCG	
11901				GGAGATGCGG CCTCTACGCC	
11951				TGAATCGCGA ACTTAGCGCT	
12001				ACCGGGATTA TGGCCCTAAT	
12051				CGCATACGAG GCGTATGCTC	
		ATTGAAAGTT	TTTTCGAAAT	TGTTGGTGCA	CGCATGCGAA
		TCCTCCACCG	ATATCCTGAC	TACGTAGACA	CCCTGAAACA
		CTCGTTTTGG	GTTTATCGTT	CGGCGAGTAC	CGCGTCGACA
12251	TCCTTATAGT AGGAATATCA	GCAGCACAGC CGTCGTGTCG	AGGGACAACG TCCCTGTTGC	AGGCATTCAG TCCGTAAGTC	GGATGCGCTG CCTACGCGAC

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12351				CTTGAGCCTG GAACTCGGAC	
12401				TGGGCAAGTT ACCCGTTCAA	
12451				GACAAGGAGG CTGTTCCTCC	
12501	••••			GCTTACCTTG CGAATGGAAC	
12551		-		AGGCCGTGAG TCCGGCACTC	
12601				CACAGCCTGC GTGTCGGACG	
12651				CGAGTCCTAC GCTCAGGATG	
12701				GCGCCCTGGA CGCGGGACCT	
12751				CGCGCTGGCA GCGCGACCGT	
12801				CGAGCCAGAG GCTCGGTCTC	
12851				GCAAGACGCA CGTTCTGCGT	
12901				CCGGCCTTAA GGCCGGAATT	
12951				TCGCTGACTG AGCGACTGAC	
13001	TGAÇGCGTTC ACTGCGCAAG	CGGCAGCAGC	CGCAGGCCAA GCGTCCGGTT	CCGGCTCTCC	GCAATTCTGG CGTTAAGACC
13051					GGTGCTGGCG CCACGACCGC
13101					ACGAGGCCGG TGCTCCGGCC
13151					AACAGCGGCA TTGTCGCCGT
13201					CGAGGCCGTG GCTCCGGCAC

Figure 26 N

13301	ACTAAACGCC	TTCCTGAGTA	CACAGCCCGC	CAACGTGCCG	CGGGGACAGG
	TGATTTGCGG	AAGGACTCAT	GTGTCGGGCG	GTTGCACGGC	GCCCCTGTCC
13351	AGGACTACAC	CAACTTTGTG	AGCGCACTGC	GGCTAATGGT	GACTGAGACA
	TCCTGATGTG	GTTGAAACAC	TCGCGTGACG	CCGATTACCA	CTGACTCTGT
13401		AGGTGTACCA			
	GGCGTTTCAC	TCCACATGGT	CAGACCCGGT	CTGATAAAA	AGGTCTGGTC
13451		CTGCAGACCG	-	* *	
		GACGTCTGGC			
13501		GGGGGTGCGG			
		CCCCCACGCC			
13551		CGCCCAACTC			
		GCGGGTTGAG			
13601					CACTTGCTGA
		CCGTCGCACA			•
13651		CGAGGCCATA			•
		GCTCCGGTAT			
13701		CAAGTGTCAG			
		GTTCACAGTC			
13751		ACCCTAAACT			
		TGGGATTTGA			
13801		CAGTTTAAAC			
12051		GTCAAATTTG			
13851		TGAGCCTTAA			-
12001		ACTCGGAATT			
13901		ATGACCGCGC			
12051	,	TACTGGCGCG			
13951		TATCAACCGC ATAGTTGGCG			
14001		•			
14001	GTGAACCCCG				TGACCGATGG
1.4054	GCCCCTGGT				
14051					
		AAGATGTGGC		•	
14101	GATTCCTCTG	-			
		CCTGCTGTAT			
14151	ACCCTGCTAG			· · · · · · · · · · · · · · · · · · ·	
	TGGGACGATC	TUAAUGTTGT	CGCGCTCGTC	CGTCTCCGCC	GCGACGCTTT

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14251		TGCTAGTAGC ACGATCATCG			
14301		CCACCCGCCC GGTGGGCGGG			
14351		CTGCAGCCGC GACGTCGGCG			
14401		GATAGAGAGC CTATCTCTCG			
14451		AGCACAGGGA TCGTGTCCCT			
14501		GACCGTCAGC CTGGCAGTCG			
14551		CAGCGTCCTG GTCGCAGGAC			
14601		CCAGGCTGGG GGTCCGACCC			
14651	ACGTTTTATT	AAAACTCACC TTTTGAGTGG	TTCCGGTACC	GTGGCTCGCA	ACCAAAAGAA
14701	CATAAGGGGA	TAGTATGCGG ATCATACGCC	GCGCGCCGCT	ACATACTCCT	TCCAGGAGGA
14751		AGAGTGTGGT TCTCACACCA			
14801	AAGAGGGAAG	GATGCTCCCC CTACGAGGGG	ACCTGGGCGG	CAAACACGGA	GGCGCCATGG
14851	ÄCGCCGGATG	CGGGGGGAGA GCCCCCTCT	TTGTCGTAGG	CAATGAGACT	CAACCGTGGG
14901	GATAAGCTGT	GGTGGGCACA	CATGGACCAC	CTGTTGTTCA	CAACGGATGT GTTGCCTACA
	CCGTAGGGAC	TTGATGGTCT	TGCTGGTGTC	GTTGAAAGAC	ACCACGGTCA TGGTGCCAGT
	AAGTTTTGTT	ACTGATGTCG	GGCCCCTCC	GTTCGTGTGT	GACCATCAAT CTGGTAGTTA
	GAACTGCTGG	CCAGCGTGAC	CCCGCCGCTG	GACTTTTGGT	TCCTGCATAC AGGACGTATG
15101	CAACATGCCA GTTGTACGGT	AATGTGAACG TTACACTTGC	AGTTCATGTT TCAAGTACAA	TACCAATAAG ATGGTTATTC	TTTAAGGCGC AAATTCCGCG

Figure 26 P

WO 02/022080				PCT/US01/28861
15151	GGGTGATGGT CCCACTACCA		ACAATCAGGT TGTTAGTCCA	
	TACGAGTGGG ATGCTCACCC	 		
15251	GACCATAGAC CTGGTATCTG		GGAGCACTAC CCTCGTGATG	
15301	GCAGACAGAA CGTCTGTCTT	 	TCGGGGTAAA AGCCCCATTT	
15351	CGCAACTTCA GCGTTGAAGT	 	ACTGGTCTTG TGACCAGAAC	
15401	GGTATATACA CCATATATGT		CATCATTTTG GTAGTAAAAC	
15451	GCGGGGTGGA CGCCCCACCT		GCAACTTGTT CGTTGAACAA	
15501	AAGCGGCAAC TTCGCCGTTG		ATCACCTACG TAGTGGATGC	
15551	GGGTGGTAAC CCCACCATTG		GGAÇGCCTAC CCTGCGGATG	
15601	TGAAAGATGA ACTTTCTAÇT		GCGCAGGCGG CGCGTCCGCC	
15651	AGTGGCAGCG TCACCGTCGC		GCGGCAGCCG CGCCGTCGGC	
15701	GCCGGTGGAG CGGCCACCTC		TCGCGGCGAC AGCGCCGCTG	
15751			AAGCAGCGGC TTCGTCGCCG	
15801	GCCCCGCTG CGGGGGCGAC			AACCGGTGAT TTGGCCACTA
15851	CAAACCCCTG GTTTGGGGAC		CAGTTACAAC GTCAATGTTG	
15901	ATGACAGCAC TACTGTCGTG		GGTACCTTGC CCATGGAACG	
15951				GCACTCCTGA CGTGAGGACT
16001	CGTAACCTGC GCATTGGACG		GTCGTTGCCA CAGCAACGGT	
16051			AGATCAGCAA TCTAGTCGTT	CTTTCCGGTG GAAAGGCCAC

Figure 26 Q

16151		TACCTCTCTG ATGGAGAGAC	
16201		GCGCGGGCC	
16251	 	 CTCACAGATC GAGTGTCTAG	
16301		GCGAGTGACC CGCTCACTGG	
16351		AGGCCCTGGG TCCGGGACCC	
16401		GCAAGCATGT CGTTCGTACA	
16451		GCGCTTCCCA CGCGAAGGGT	
16501		ACCCAGTGCG TGGGTCACGC	
16551		CGCGGCCGCA GCGCCGGCGT	
16601		GGAGGAGGCG CCTCCTCCGC	
16651		ACGCGGCCAT TGCGCCGGTA	
16701		AAGAGACGGC TTCTCTGCCG	
16751		TGCCGCCCAA ACGGCGGGTT	
16801		GCCGACGGGC CGGCTGCCCG	
16851			CCAGGTCCAG GGTCCAGGTC
16901			ATGACTCAGG TACTGAGTCC
16951			CGGCCTGCGC
17001			GAAAAAACTA CTTTTTTGAT

7igure 26 R

17101			AAAGAAGAGA TTTCTTCTCT		
17151			GAAGGAAGAG CTTCCTTCTC		
17201	GCTAAAGCGG CGATTTCGCC		AAAAGAAAGA TTTTCTTTCT		
17251			GCTACCGCGC CGATGGCGCG		
17301		• • • • • • • •	TGTTTTGCGA ACAAAACGCT		
17351			CCCGCACCTA GGGCGTGGAT		
17401			CTTGAGCAGG GAACTCGTCC		
17451			TAAGGACATG ATTCCTGTAC		
17501			TAAAGCCCGT ATTTCGGGCA		
17551			GAAAAGCGCG CTTTTCGCGC		
17601			GCTGATGGTA CGACTACCAT		
17651	AGATGTCTTG TCTACAGAAC	GAAAAAATGA .CTTTTTTACT	CCGTGGAACC GGCACCTTGG	TGGGCTGGAG ACCCGACCTC	CCCGAGGTCC GGGCTCCAGG
17701			GTGGCGCCGG CACCGCGGCC		
17751	GACGTTCAGA CTGCAAGTCT	TACCCACTAC ATGGGTGATG	CAGTAGCACC GTCATCGTGG	AGTATTGCCA TCATAACGGT	CCGCCACAGA GGCGGTGTCT
17801	GGGCATGGAG CCCGTACCTC				GCGGATGCCG CGCCTACGGC
17851	CGGTGCAGGC				GGAGGTGCAA CCTCCACGTT
17901	ACGGACCCGT TGCCTGGGCA				CGCGCCGTTC
17951	GAGGAAGTAC CTCCTTCATG				GCCCTACATC CGGGATGTAG

Figure 265

18051	AGACGAGCAA TCTGCTCGTT	CTACCCGACG GATGGGCTGC		
18101		CAGCCCGTGC GTCGGGCACG		
18151		CAGGACCCTG GTCCTGGGAC		
18201		AGCCGGTCTT TCGGCCAGAA		
18251		TTCCCGGTGC AAGGGCCACG		
18301		CGGCCACGGC GCCGGTGCCG		
18351		GCGCGTCGCA CGCGCAGCGT		
18401		CTGATCGCCG GACTAGCGGC		
18451		GCAGGCGCAG CGTCCGCGTC		
18501		ATAAAAAGTC TATTTTTCAG		
18551	-	AATGGAAGAC TTACCTTCTG		
18601		CGTTCATGGG GCAAGTACCC		
18651		GCCTTCAGCT CGGAAGTCGA		
18701		CGTTAAGAAC GCAATTCTTG		CAGCAGCACA GTCGTCGTGT
18751				AACAAAAGGT TTGTTTTCCA
18801				CTGGCCAACC GACCGGTTGG
18851				CCCTCCCGTA GGGAGGGCAT
18901				GGCGTGGCGA CCGCACCGCT

Figure 26.T

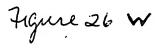
19001		GTACGAGGAG CATGCTCCTC			
19051		CCATGGCTAC GGTACCGATG			
19101		CCTCCCCCG GGAGGGGGGC			
19151		CGTTGTTGTA GCAACAACAT			
19201		GTCCGCGATC CAGGCGCTAG			
19251		AACAGCATCG TTGTCGTAGC			
19301		CTGATAGCTA GACTATCGAT			
19351		CAGAGGAGCT GTCTCCTCGA			
19401		TTCGATGATG AAGCTACTAC			
19451		CGGAGTACCT GCCTCATGGA			
19501		TACTTCAGCC ATGAAGTCGG			
19551	GCGGATGCGT	CGACGTGACC	TGTCTGGCCA	GGGTCGCAAA	CTGCGACGCC
19601	AAGTAGGGAC	TGGACCGTGA ACCTGGCACT	CCTATGACGC	ATGAGCATGT	TCCGCGCCAA
19651	GTGGGATCGA	GTGGGTGATA CACCCACTAT	TGGCACACGA	CCTGTACCGA	AGGTGCATGA
	AACTGTAGGC	GCCGCACGAC	CTGTCCCCGG	GATGAAAATT	GCCCTACTCT CGGGATGAGA
19751					ATCCTTGCGA TAGGAACGCT
19801					GAAGAGGACG CTTCTCCTGC
19851					AAAAACTCAC TTTTTGAGTG

Figure 26 U

19951	TCAAATAGGT AGTTTATCCA	 		
20001	AACCTGAACC TTGGACTTGG	GAATCTCAGT CTTAGAGTCA		
20051		 AAAAAAGACT TTTTTTCTGA		
20101		CAAATGAAAA GTTTACTTTT		
20151	TAAAGCAACA ATTTCGTTGT	CTAGAAAGTC GATCTTTCAG		
20201		AGGCAATGGT TCCGTTACCA		
20251		TAGATATAGA ATCTATATCT		
20301		 GAAGGTAACT CTTCCATTGA		
20351		 TAATTACATT ATTAATGTAA		
20401		GCACGGGTAA CGTGCCCATT	,	
20451		GTTGTAGATT CAACATCTAA		
20501		 TGATTCCATT ACTAAGGTAA		
20551		TTGACAGCTA AACTGTCGAT		GTTAGAATTA CAATCTTAAT
20601		 GATGAACTTC CTACTTGAAG		
20651	GGÄGGTGTGA CCTCCACACT			CTAAAACAGG GATTTTGTCC
	TCAGGAAAAT AGTCCTTTTA			
20751	AAATAAGAGT TTTATTCTCA			AAATGCCAAC TTTACGGTTG
20801				TGCCCGACAA ACGGGCTGTT

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20901			-	CCGGGCTAGT GGCCCGATCA	
20951		· - · -		TATATGGACA ATATACCTGT	
21001				CTACCGCTCA GATGGCGAGT	
21051				AGGTGCCTCA TCCACGGAGT	
21101	· ·			TCATACACCT AGTATGTGGA	
21151		_		GAGCTCCCTA CTCGAGGGAT	
21201			•	ATAGCATTTG TATCGTAAAC	
21251				TCCACGCTTG AGGTGCGAAC	
21301				CGACTATCTC GCTGATAGAG	
21351				CCAACGTGCC GGTTGCACGG	
21401				TGGGCCTTCA ACCCGGAAGT	
21451				CTACGACCCT GATGCTGGGA	
21501				CCTTTTACCT GGAAAATGGA	
21551				TCTGTCAGCT AGACAGTCGA	
21601	TGACCGCCTG ACTGGCGGAC	-	•	AATTAAGCGC TTAATTCGCG	
21651	GGGAGGGTTA CCCTCCCAAT			TGACCAAAGA ACTGGTTTCT	
21701	GTACAAATGC CATGTTTACG			TACCAGGGCT ATGGTCCCGA	
21751	AGAGAGCTAC TCTCTCGATG			CTTTAGAAAC GAAATCTTTG	



21851	GGCATCCTAC CCGTAGGATG		CAACTCTGGA GTTGAGACCT		
21901	GTGGTACGCG	CTTCCTGTCC	CCTACCCTGC GGATGGGACG	ATTGAAGGGG	ATAGGCGAAT
21951	ATCCGTTCTG	GCGTCAACTG	AGCATTACCC TCGTAATGGG	TCTTTTTCAA	AGAAACGCTA
22001	GCGTGGGAAA	CCGCGTAGGG	ATTCTCCAGT TAAGAGGTCA	TTGAAATACA	GGTACCCGCG
22051	TGAGTGTCTG	GACCCGGTTT	ACCTTCTCTA TGGAAGAGAT	GCGGTTGAGG	CGGGTGCGCG
22101	ATCTGTACTG	AAAACTCCAC	GATCCCATGG CTAGGGTACC	TGCTCGGGTG	GGAAGAAATA
22151	CAAAACAAAC	TTCAGAAACT	CGTGGTCCGT GCACCAGGCA	CACGTGGTCG	GCGTGGCGCC
22201	GCAGTAGCTT	TGGCACATGG	TGCGCACGCC ACGCGTGCGG	GAAGAGCCGG	CCGTTGCGGT
22251	GTTGTATTTC	TTCGTTCGTT	CATCAACAAC GTAGTTGTTG ATTGTCAAAG	TCGACGGCGG	TACCCGAGGT
22301	CACTCGTCCT	TGACTTTCGG	TAACAGTTTC GCGCTTTCCA	TAGAACCAAC	ACCCGGTATA
22351	AAAAACCCGT	GGATACTGTT	CGCGAAAGGT ATACGGCCGG	CCGAAACAAA	GAGGTGTGTT
22401	CGAGCGGACG	CGGTATCAGT	TATGCCGGCC	AGCGCTCTGA	CCCCCGCATG
22451	TGACCTACCG	GAAACGGACC	TTGGGCGTGA	GTTTTTGTAC	
22501	CTCGGGAAAC	CGAAAAGACT	GGTCGCTGAG	TTCGTCCAAA	TGGTCAAACT
		GAGGACGCGG	CATCGCGGTA	ACGAAGAAGG	GGGCTGGCGA
	CATATTGCGA	CCTTTTCAGG	TGGGTTTCGC	ATGTCCCCGG	GTTGAGCCGG CCAACTGGCC
	CGGACACCTG	ATAAGACGAC	GTACAAAGAG	GTGCGGAAAC	GGTTGACCGG
22701					ACCGGGGTAC TGGCCCCATG

Figure 26 X

22801		TCTACAGCTT AGATGTCGAA			
22851		CAGATTAGGA GTCTAATCCT			
22901	TGTAAAAATA ACATTTTTAT	ATGTACTAGA TACATGATCT	GACACTTTCA CTGTGAAAGT	ATAAAGGCAA TATTTCCGTT	ATGCTTTTAT TACGAAAATA
22951		TCGGGTGATT AGCCCACTAA			
23001	TTAAAAATCA AATTTTTAGT	AAGGGGTTCT TTCCCCAAGA			
23051	ACACGTTGCG TGTGCAACGC	ATACTGGTGT TATGACCACA	TTAGTGCTCC AATCACGAGG	ACTTAAACTC TGAATTTGAG	AGGCACAACC TCCGTGTTGG
23101	ATCCGCGGCA TAGGCGCCGT	GCTCGGTGAA CGAGCCACTT	GTTTTCACTC CAAAAGTGAG	CACAGGCTGC GTGTCCGACG	GCACCATCAC CGTGGTAGTG
23151		AGCAGGTCGG TCGTCCAGCC			
23201	CTCCGCCCTG GAGGCGGGAC	CGCGCGCGAG GCGCGCGCTC	TTGCGATACA AACGCTATGT	CAGGGTTGCA GTCCCAACGT	GCACTGGAAC CGTGACCTTG
23251		CCGGGTGGTG GGCCCACCAC			
23301		TCCAGGTCCT AGGTCCAGGA			
23351		CCTTCCCAAA GGAAGGGTTT			TGAGTTGCAC ACTCAACGTG
23401	TCGCACCGTA AGCGTGGCAT	GTGGCATCAA CACCGTAGTT	AAGGTGACCG TTCCACTGGC	TGCCCGGTCT ACGGGCCAGA	GGGCGTTAGG CCCGCAATCC
23451	ATACAGCGCC TATGTCGCGG	TGCATAAAAG ACGTATTTTC	CCTTGATCTG GGAACTAGAC	CTTAAAAGCC GAATTTTCGG	ACCTGAGCCT TGGACTCGGA
23501	TTGCGCCTTC AACGCGGAAG	AGAGAAGAAC TCTCTTCTTG	ATGCCGCAAG TACGGCGTTC	ACTTGCCGGA TGAACGGCCT	AAACTGATTG TTTGACTAAC
23551	GCCGGACAGG CGGCCTGTCC	CCGCGTCGTG GGCGCAGCAC	CACGCAGCAC GTGCGTCGTG	CTTGCGTCGG GAACGCAGCC	TGTTGGAGAT ACAACCTCTA
23601	CTGCACCACA GACGTGGTGT	TTTCGGCCCC	ACCGGTTCTT TGGCCAAGAA	CACGATCTTG GTGCTAGAAC	GCCTTGCTAG CGGAACGATC
23651	ACTGCTCCTT TGACGAGGAA	CAGCGCGCGC	TGCCCGTTTT ACGGGCAAAA	CGCTCGTCAC GCGAGCAGTG	ATCCATTTCA TAGGTAAAGT

Figure 26 Y

WO 02/022080			•	PCT/US01/28861
23701	ATCACGTGCT TAGTGCACGA	CATAATGCTT GTATTACGAA		
23751	GCCTTCGATC CGGAAGCTAG	GGTGCAGCCA CCACGTCGGT		
23801	CGTGATGCTT GCACTACGAA	TCTGCAAACG AGACGTTTGC		
23851	AATCGCCCCA TTAGCGGGGT	AAAGGTCTTG TTTCCAGAAC		
23901		TCAGCCAGGT AGTCGGTCCA		
23951	CTTCCACTTG GAAGGTGAAC	AGTTTGAAGT TCAAACTTCA		
24001	ACGTGGTACT TGCACCATGA	 CGCGCGCGCA GCGCGCGCGT		
24051		 TCAGCGGGTT AGTCGCCCAA		
24101		TCTTCCTCTT AGAAGGAGAA		
24151	ACTGGGTCGT TGACCCAGCA	 CCGCCGCACT GGCGGCGTGA		
24201		GGTTGCTGAA CCAACGACTT		
24251		CTGTCCACGA GACAGGTGCT		
24301		 GCGCTTCTTT CGCGAAGAAA		
	CAAATCCGCC GTTTAGGCGG			·
24401	GCGCGTCTTG CGCGCAGAAC	 TCCTCGTCCT AGGAGCAGGA		
24451.	ATCCGCTTTT TAGGCGAAAA	CCGGGGAGGC GGCCCCTCCG	-	
24501	CGACACGTCC GCTGTGCAGG	GGGGACGTCG CCCCTGCAGC		
24551	CGGGGGTGGT GCCCCCACCA	TCCTCTTCCC AGGAGAAGGG		
24601	TATAGGCAGA ATATCCGTCT	GGAGTCAGTC CCTCAGTCAG		

Figure 262

24701			TTGAGGAGGA AACTCCTCCT	
24751		 	GACGACGAGG CTGCTGCTCC	
24801		 	CAACGCAGAG GTTGCGTCTC	
24851	AACAAGTCGG TTGTTCAGCC		GCGACTACCT CGCTGATGGA	
24901		 	CAGTGCGCCA GTCACGCGGT	
24951		 	CGCCATAGCG GCGGTATCGC	
25001		 	GCGTACCCCC CGCATGGGGG	
25051			CTCAACTTCT GAGTTGAAGA	
25101			CATCTTTTC GTAGAAAAAG	
25151		 	GCCGAGCGGA CGGCTCGCCT	
25201			ATCGCCTCGC TAGCGGAGCG	
25251			CGAGAAGCGC GCTCTTCGCG	
25301			GTCACTCTGG CAGTGAGACC	
25351			GTACTAAAAC CATGATTTTG	
25401	GGTCACCCAC CCAGTGGGTG			AAGGTCATGA TTCCAGTACT
25,451	GCACAGTCAT CGTGTCAGTA		GTGCGCAGCC CACGCGTCGG	
25501	GATGCAAATT CTACGTTTAA			CAGTTGGCGA GTCAACCGCT
25551	CGAGCAGCTA GCTCGTCGAT			GACTTGGAGG CTGAACCTCC

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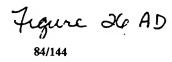
25651		GGTTCTTTGC CCAAGAAACG			
25701	AACATTGCAC TTGTAACGTG	TACACCTTTC ATGTGGAAAG			
25751		GGAGCTCTGC CCTCGAGACG		• •	
25801	GAAAACCGCC CTTTTGGCGG	TTGGGCAAAA AACCCGTTTT			
25851		TACGTCCGCG ATGCAGGCGC			
25901		CATGGGCGTT GTACCCGCAA			
25951		AGAAACTGCT TCTTTGACGA			
26001		CGCTCCGTGG GCGAGGCACC	•		
26051		TAAAACCCTG ATTTTGGGAC			
26101.	AGCATGTTGC TCGTACAACG	AGAACTTTAG TCTTGAAATC			
26151		TGCTGTGCAC ACGACACGTG		•	
26201		TCCGCCGCTT AGGCGGCGAA			
26251		CCTACCACTC GGATGGTGAG			
26301		TGTCACTGTC ACAGTGACAG			CACCGCTCCC GTGGCGAGGG
26351					CGGTACCTTT GCCATGGAAA
26401					CGGGGTTGAA GCCCCAACTT
26451					TTTGTACCTG AAACATGGAC
26501					ATCCCGCCCG TAGGGCGGGC

Figure 26 AB

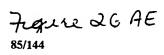
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WO 02/022080		> 00mm> 0000	00000000000	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	PCT/US01/28861
26551	CCTAATGCGG				TGTAAGAACC
	GGATTACGCC	1CGAN1GGCG	GACGCAGIAA	166616666	101AAGAACC .
26601	CCAATTGCAA	GCCATCAACA	AAGCCCGCCA	AGAGTTTCTG	CTACGAAAGG
	GGTTAACGTT	CGGTAGTTGT	TTCGGGCGGT	TCTCAAAGAC	GATGCTTTCC
26651	GACGGGGGGT				
	CTGCCCCCCA	AATGAACCTG	GGGGTCAGGC	CGCTCCTCGA	GTTGGGTTAG
26701	CCCCGCCGC	CGCAGCCCTA	TCAGCAGCAG	CCGCGGGCCC	TTGCTTCCCA
20.02			AGTCGTCGTC		
•		•			
26751	GGATGGCACC				
	CCTACCGTGG	GTTTTTCTTC	GACGTCGACG	GCGGCGGTGG	GTGCCTGCTC
26801	GAGGAATACT	GGGACAGTCA	GGCAGAGGAG	GTTTTGGACG	AGGAGGAGGA
20001			CCGTCTCCTC		
26851	GGACATGATG	GAAGACTGGG	AGAGCCTAGA	CGAGGAAGCT	TCCGAGGTCG
	CCTGTACTAC	CTTCTGACCC	TCTCGGATCT	GCTCCTTCGA	AGGCTCCAGC
26001	AAGAGGTGTC	NCNCCNNNCN		ででででできませ	CCCCTCCCC
26901			GGCAGTGGGA		
	Ticiccheno	10.0011.01			
26951	GCGCCCCAGA	AATCGGCAAC	CGGTTCCAGC	ATGGCTACAA	CCTCCGCTCC
	CGCGGGGTCT	TTAGCCGTTG	GCCAAGGTCG	TACCGATGTT	GGAGGCGAGG
27001	TCAGGCGCCG				
	AGTCCGCGGC	GGCCGTGACG	GGCAAGCGGC	TGGGTTGGCA	TCTACCCTGT
27051	CCACTGGAAC	CAGGGCCGGT	AAGTCCAAGC	AGCCGCCGCC	GTTAGCCCAA
•			TTCAGGTTCG		
27101	GAGCAACAAC				
	CTCGTTGTTG	TCGCGGTTCC	GATGGCGAGT	ACCGCGCCCG	TGTTCTTGCG
27151	CATAGTTGCT	TECTTECALE	ACTGTGGGG	СААСАТСТСС	TTCGCCCGCC
2/131	-		TGACACCCCC		
	0				
27201	GCTTTCTTCT				
	CGAAAGAAGA	GATGGTAGTG	CCGCACCGGA	AGGGGGCATT	GTAGGACGTA
22251	TACTACCGTC	>momom>C>C	CCC> TO CTCC	***************************************	CCCCACCAA
. 27251			GGGTATGACG		
	AIGAIGGCAG	INGNONIGIC	GGGIAIGACG	10000000001	cocco
27301	CAGCAGCGGC	CACACAGAAG	CAAAGGCGAC	. CGGATAGCAA	GACTCTGACA
	GTCGTCGCCG	GTGTGTCTTC	GTTTCCGCTG	GCCTATCGTT	CTGAGACTGT
27351	AAGCCCAAGA				
	TTCGGGTTCT	TTAGGTGTCG	CCGCCGTCGT	CGTCCTCCTC	CICGCGACGC
27401	TCTGGCGCCC	AACGAACCCG	TATCGACCCG	CGAGCTTAGA	AACAGGATTT
27401					TTGTCCTAAA
27451	TTCCCACTCT				
	AAGGGTGAGA	CATACGATAT	AAAGTTGTCT	CGTCCCCGGT	TCTTGTTCTC

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27551		GAAGATCAGC CTTCTAGTCG	 •	-
27601		ATACTGCGCG TATGACGCGC		
27651		AAGCGCGAAA TTCGCGCTTT	 	
27701	•••••	GTTGTCAGCG CAACAGTCGC	 	
27751		TTACCAGCCA AATGGTCGGT	 	
27801		CCCGAATAAA GGGCTTATTT		
27851		GGAATACGCG CCTTATGCGC		
27901		CACCACACCT GTGGTGTGGA		
27951		TGTACCAGGA ACATGGTCCT		
28001		CAGGCCGAAG GTCCGGCTTC		
28051		TCGTCACAGG AGCAGTGTCC		
28101		GAGGGCGAGG CTCCCGCTCC		
28151		CTCCGTCCGG GAGGCAGGCC		
28201		CACGCCTCGT GTGCGGAGCA		
28251				TTGAGGAGTT AACTCCTCAA
28301				GGCCACTATC CCGGTGATAG
28351				GGCGGACGGC CCGCCTGCCG
28401				TGAAACACCT ACTTTGTGGA



WO 02/022080					PCT/US01/28861
20451	GGTCCACTGT	00000000	» CDCCDDDCC	CCCCACTCC	ርርጥር እርጥ ማጥጥ
28451				GGCGCTGAGG	
	CCAGGTGACA	GCGGCGG1G1	TCACGAAACG	GGCGC 1 GAGG	CCACTCAAAA
20501	GCTACTTTGA	> DDCCCCC > C	CARCARATCC	ACCCCCCCCC	CCACCCCCCC
28501				TCCCGGGCCG	
	CGATGAAACT	TAACGGGCTC	CIAGIAIAGC	100000000	CGIGCCGCAG
20553	CGGCTTACCG	CCCACCCACA		אכררייניאייזייר	CCCACTTAC
28331				TCGGACTAAG	
	GCCGAATGGC	GGGICCCICI	CUANCUGUCA	ICOGNCIANO	CCCTCAAATG
20601	CCAGCGCCCC	COCCUACOTO	ACCCCCACAC	CCCACCCTCT	CTTCTCACTC
28601				CCCTGGGACA	
	GGTCGCGGG	GACGATCAAC	1000001010	CCCIGGGACA	CAAGAGIGAC
20651	TGATTTGCAA	ריזיביזיריריזי ארי	ССТССАТТАС	ATCAAGATCT	TTCTTCCCAT
26031				TAGTTCTAGA	
	ACTAAACGTT	GACAGGATIG	GGACCIANIG	Indiicinan	Bichicotti
20701	CTCTGTGCTG	אכדמדממדממ	ATACAGAAAT	ТАЛАДТАТАС	TGGGGCTCCT
20701				ATTTTATATG	
	GAGACACGAC	icarattat.	IMIOICIII.		,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,
22751	ATCGCCATCC	TCTAAACCCC	ACCGTCTTCA	CCCGCCCAAG	CAAACCAAGG
20751				GGGCGGGTTC	
•	100001000			***************************************	
28801	CGAACCTTAC	CTGGTACTTT	TAACATCTCT	CCCTCTGTGA	TTTACAACAG
20001				GGGAGACACT	
		3			
28851	TTTCAACCCA	GACGGAGTGA	GTCTACGAGA	GAACCTCTCC	GAGCTCAGCT
				CTTGGAGAGG	
	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,				
28901	ACTCCATCAG	AAAAAACACC	ACCCTCCTTA	CCTGCCGGGA	ACGTACGAGT
				GGACGGCCCT	
	•				
28951	GCGTCACCGG	CCGCTGCACC	ACACCTACCG	CCTGACCGTA	AACCAGACTT
	CGCAGTGGCC	GGCGACGTGG	TGTGGATGGC	GGACTGGCAT	TTGGTCTGAA
29001	TTTCCGGACA				
	AAAGGCCTGT	CTGGAGTTAT	TGAGACAAAT	GGTCTTGTCC	TCCACTCGAA
29051				GCAGCTACTG	
	TCTTTTGGGA	ATCCCATAAT	CCGGTTTCCG	CGTCGATGAC	ACCCCAAATA
29101					TTCTCTAGAA
	CTTGTTAAGT	TCGTTGAGAT	GCCCGATAAG	ATTAAGTCCA	AAGAGATCTT
					mcoma ma con a
29151					TCTTATACTA
	AGCCCCAACC	CCAATAAGAG	ACAGAACACT	AAGAGAAATA	AGAATATGAT
		000m) \ 000m	00000000000	· ·	TTTGCATTTA
29201					AAACGTAAAT
	TGCGAAGAGA	CGGATTCCGA	OCOGCODACO	ACACACG161	PUNCATURA
20251		יייט או אייטערע אייטערערערערערערערערערערערערערערערערערערע	GGGGTCGCCx	CCCTTCTCT	TTAGGTACAT
29251	1.10.1CACC1.1	TITAMACACI	CCCCA	− CCC™GV1 GV	AATCCATGTA
	WYCHO I COWN	NAMILI I GCGA	CCCCAGCGG1	Journal	
	እ አጥ ር ርጥአርርጥ	יוידי≱ריויר ≱רירר	תתכרניתר צ ובר	CCACGGTACC	ACCCAAAAGG
29301					TGGGTTTTCC
	TINGGATECA		ANCOCAG: CO		
20251	ጥርር ልጥጥጥ ል ል	GGAGCCAGCC	ТСТААТСТТА	CATTCGCAGC	TGAAGCTAAT
23331	ברבו ברבים ב	CCTCGGTCGG	ACATTACAAT	GTAAGCGTCG	ACTTCGATTA
	STATE TARREST				



29451		AACAAAATTG TTGTTTTAAC	 	
29501		TACAGAGTAT ATGTCTCATA	 -	
29551	AAAACTTTTA TTTTGAAAAT	TGTATACTTT ACATATGAAA	 	
29601		AAACAGTATA TTTGTCATAT		
29651		TTTCTGCTGC AAAGACGACG		
29701		TACTCTATAT ATGAGATATA	 	
29751		ATGCCTTAAT TACGGAATTA		
29801		CTCGCTGCTT GAGCGACGAA	 	· · · ·
29851		GATTTAAACC CTAAATTTGG		
29901 -	CCTGAACAAT GGACTTGTTA	TGACTCTATG ACTGAGATAC	 	
29951		CTGGATGTCA GACCTACAGT		
30001		CAGTCCAACT GTCAGGTTGA		
30051		CGCCGGCGCC	 	
30101		TGCCTTTGTC ACGGAAACAG		
30151				GGCTCATCTG CCGAGTAGAC
30201	CTGCCTAAAG GACGGATTTC	CGCAAACGCG GCGTTTGCGC		
30251				ACTGAAACAC TGACTTTGTG
30301				TCCTCGAGTT AGGAGCTCAA

Figure 26 AF

30401		CACATCGAAG GTGTAGCTTC			
30451		ATTTGTCACC TAAACAGTGG			
30501		TTATCCAGTG AATAGGTCAC			
.30551		CATCCCCAGT GTAGGGGTCA			
30601		ATTATGAAAT TAATACTTTA			
30651		GTTTTGTTCC CAAAACAAGG			
30701		CTCGTATATG GAGCATATAC			
30751		GAAGCCTGGT CTTCGGACCA			
30801		CTTAGCCCTA GAATCGGGAT			
30851		ATGCCATGAA TACGGTACTT			
30901		CAAGTTGTTG GTTCAACAAC			
30951		TCCCACCCCC AGGGTGGGGG			
31001		GACACCCTAG CTGTGGGATC			
31051	AGCGCCTGCT TCGCGGACGA	AGAAAGACGC TCTTTCTGCG	AGGGCAGCGG TCCCGTCGCC	CCGAGCAACA GGCTCGTTGT	GCGCATGAAT CGCGTACTTA
31101					GGGGTATCTT CCCCATAGAA
31151	TTGTCTCGTA AACAGAGCAT				ACCACCGGAC TGGTGGCCTG
31201					GGTGGTCATG CCACCAGTAC
31251					AAACCGAAGG TTTGGCTTCC



31351		GATCTTATTC CTAGAATAAG	
31401		TAAAATCAGT ATTTTAGTCA	
31451		 CCCTCCTCCC	
31501		CCACAATCTA GGTGTTAGAT	
31551		 CCACTATCTT GGTGATAGAA	
31601		ACCTTCAACC TGGAAGTTGG	
31651		 GCCTTTTCTT CGGAAAAGAA	
31701		CCCCTGGGGT GGGGACCCCA	=
31751		 GGCATGCTTG CCGTACGAAC	
31801		CAACCTTACC GTTGGAATGG	
31851		CCAAGTCAAA GGTTCAGTTT	
31901		GAAGCCCTAA CTTCGGGATT	
31951		ACTCACCATG TGAGTGGTAC	
32001		GCATTGCCAC CGTAACGGTG	
32051	CAGAAGGAAA GTCTTCCTTT	CAAACATCAG GTTTGTAGTC	
32101	AGCAGTACCC TCGTCATGGG	TGCCTCACCC ACGGAGTGGG	
32151	TAGCTTGGGC ATCGAACCCG	 AAGAGCCCAT TTCTCGGGTA	
32201	TAGGACTAAA ATCCTGATTT	CCTTTGCATG GGAAACGTAC	

Figure 26 AH

32301 ·	AACTAAAGTT TTGATTTCAA		TTCACAAGGC AAGTGTTCCG	
32351		 	CTCAAAACAG GAGTTTTGTC	
32401		-	AACCAACTAA TTGGTTGATT	
32451			CCACAACTTG GGTGTTGAAC	
32501		 	CAAACAATTC GTTTGTTAAG	-
32551	•	 -	ATGTTTGACG TACAAACTGC	
32601		 	TGGTTCACCT ACCAAGTGGA	
32651	ACACAAATCC TGTGTTTAGG		ATGGCCTAGA TACCGGATCT	
32701	,	 	GGCCTTAGTT CCGGAATCAA	
32751			TGATAAGCTA ACTATTCGAT	
32801	• • • • • • • • • •	 	TAAATGCAGA ATTTACGTCT	
32851	AAACTCACTT TTTGAGTGAA		AGTCAAATAC TCAGTTTATG	
32901			TCCAATATCT AGGTTATAGA	
32951		 		GCTACTAAAC CGATGATTTG
33001	AATTCCTTCC TTAAGGAAGG			GAGATCTTAC CTCTAGAATG
33051	TGAAGGCACA ACTTCCGTGT			AACCTATCAG TTGGATAGTC
33101	CTTATCCAAA GAATAGGTTT			TGTCAGTCAA ACAGTCAGTT
33151	GTTTACTTAA CAAATGAATT			CCATTACACT GGTAATGTGA

Figure 26 AI 89/144

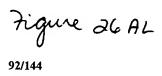
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33301		ACACTTTTTC TGTGAAAAAG		
33351		CAACGTGTTT GTTGCACAAA		
33401		GTAGTATAGC CATCATATCG		
33451	•	CAAACTCACA GTTTGAGTGT		
33501		AGAGTACACA TCTCATGTGT		
33551		GGGTAACAGA CCCATTGTCT		
33601		GCCAAACGCT CGGTTTGCGA		
33651		GTTCATGTCG CAAGTACAGC		
33701		GTTGCTTAAC CAACGAATTG		
33751	• • • • • • • • • • • • • • • • • • • •	TCATAATCGT AGTATTAGCA	 •	
33801		AAACTGCTGC TTTGACGACG		
33851		TCTCCTCAGC AGAGGAGTCG		GCATAAGGCG CGTATTCCGC
33901			 	AAATCAGCAC TTTAGTCGTG
33951				ACAGTGCAAG TGTCACGTTC
34001				CGTGGCCATC GCACCGGTAG
34051				AACACGCTGG TTGTGCGACC
34101				CTCCCGGTAC GAGGGCCATG

Figure 26 AJ

34201		ACCTGCCCGC TGGACGGGCG		
34251		GTGGAGAGCC CACCTCTCGG		
34301		CAATGTTGGC GTTACAACCG		
34351		AGCTCCTCCC TCGAGGAGGG		
34401		CAGCGTAAAT GTCGCATTTA		
34451		GCATTGTCAA CGTAACAGTT		
34501		GTAGCGCGGG CATCGCGCCC		
34551		AGTGCGCCGA TCACGCGGCT		
	ATGCCAAATG . TACGGTTTAC			
34651		ACAAACAGAT TGTTTGTCTA		
34701		AGTTGTAGTA TCAACATCAT	 	
34751		GGTTCTATGT CCAAGATACA		
34801		CGCAGAATAA GCGTCTTATT		
34851		ACACGGGAGG TGTGCCCTCC		
34901				ATCTATTAAG TAGATAATTC
34951	TGAACGCGCT ACTTGCGCGA	CCCCTCCGGT GGGGAGGCCA		
35001	GATAATGGCA CTATTACCGT			AGGCAAACGG TCCGTTTGCC
35051	CCCTCACGTC GGGAGTGCAG			GTGAATCTCC CACTTAGAGG

Figure 26 AK

35151				CCGAATATTA GGCTTATAAT	
35201				CCTTCAGCCT GGAAGTCGGA	
35251				AGACCTGTAT TCTGGACATA	-
35301				CGTAGGTCCC GCATCCAGGG	
35351				GACCAGCGCG CTGGTCGCGC	
35401				TGATTATGAC ACTAATACTG	
35451				TAAGCTTGTT ATTCGAACAA	
35501				ATCAGGCAAA TAGTCCGTTT	
35551				GCAGATAAAG CGTCTATTTC	
35601				TTTCTCTCAA AAAGAGAGTT	
35651				CAAAAAAACA GTTTTTTTGT	
35701	•			CCTTATAAGC GGAATATTCG	
35751		-	-	ACTGGTCACC TGACCAGTGG	
35801				GGAGTCATAA CCTCAGTATT	TGTAAGACTC ACATTCTGAG
35851					AAGCGACCGA TTCGCTGGCT
35901					CATTACAGCC GTAATGTCGG
35951					CATAAACACC GTATTTGTGG
36001					TCCAGAACAA AGGTCTTGTT



36101				ACACGGCACC TGTGCCGTGG	
36151				GCGAGTATAT CGCTCATATA	
36201	AAAATGACGT TTTTACTGCA			AACACCCAGA TTGTGGGTCT	
36251				AACCCACAAC TTGGGTGTTG	
36301				TTCCCATTTT AAGGGTAAAA	
36351				CTAAAACCTA GATTTTGGAT	
36401				ACTCCACCCC TGAGGTGGGG	
		•			PacI
36451	TATTGGCTTC	*****	አ እርርጥአጥአጥጥ		TTAATTAACA
36451				TAACTACTAC	
36501	<u>አ</u> ጥተርርርልጥርጥ	GCGACGCGAG	GCTGGATGGC	CTTCCCCATT	ATGATTCTTC
30301				GAAGGGGTAA	
36551				TGCAGGCCAT ACGTCCGGTA	
36601				CAAGGCCAGC GTTCCGGTCG	
36651				TTTCCATAGG AAAGGTATCC	
36701				GTCAGAGGTG CAGTCTCCAC	
36751	ACAGGACTAT TGTCCTGATA			CCTGGAAGCT GGACCTTCGA	
36801	CTCTCCTGTT GAGAGGACAA			ATACCTGTCC TATGGACAGG	
36851	CTTCGGGAAG GAAGCCCTTC			CACGCTGTAG GTGCGACATC	
36901	TCGGTGTAGG AGCCACATCC				AACCCCCCGT TTGGGGGGCA

Figure 26 AM

37001		 	CAGCCACTGG GTCGGTGACC	
37051		 	GAGTTCTTGA CTCAAGAACT	
37101		 	TGGTATCTGC ACCATAGACG	
37151		 	GCTCTTGATC CGAGAACTAG	
37201		 	TGCAAGCAGC ACGTTCGTCG	
37251	•	 	GATCTTTTCT CTAGAAAAGA	
37301			GGATTTTGGT CCTAAAACCA	
37351		 	AATCAATCTA TTAGTTAGAT	• "
37401		 	TTAATCAGTG AATTAGTCAC	
37451			AGTTGCCTGA TCAACGGACT	
37501			CATCTGGCCC GTAGACCGGG	
37551		 	CCAGATTTAT GGTCTAAATA	
37601		•	TGGTCCTGCA ACCAGGACGT	
37651		 	AAGCTAGAGT TTCGATCTCA	_
37701	CCAGTTAATA GGTCAATTAT			GCATCGTGGT CGTAGCACCA
37751	GTCACGCTCG CAGTGCGAGC		CAGCTCCGGT GTCGAGGCCA	
37801	CAAGGCGAGT GTTCCGCTCA	 	GCAAAAAAGC CGTTTTTTCG	
37851	TTCGGTCCTC AAGCCAGGAG		•	TGTTATCACT ACAATAGTGA

Figure 26 AN.

37951	GATGCTTTTC CTACGAAAAG				
38001	TGTATGCGGC ACATACGCCG				
38051	CGCGCCACAT GCGCGGTGTA		TAAAAGTGCT ATTTTCACGA		
38101	CGGGGCGAAA GCCCCGCTTT		ATCTTACCGC TAGAATGGCG		
38151	TAACCCACTC ATTGGGTGAG	GTGCACCCAA CACGTGGGTT	CTGATCTTCA GACTAGAAGT	GCATCTTTTA CGTAGAAAAT	CTTTCACCAG GAAAGTGGTC
38201			CAGGAAGGCA GTCCTTCCGT		
38251			TGAATACTCA ACTTATGAGT		
38301			TTATTGTCTC AATAACAGAG		
38351			AAATAGGGGT TTTATCCCCA		
38401			GAAACCATTA CTTTGGTAAT		
38451	AAAAATAGGC TTTTTATCCG		GCCCTTTCGT CGGGAAAGCA		
		PacI			

38501 TTCTTAATTT CTTAATTAA (SEQ ID NO:32) AAGAATTAAA GAATTAATT (SEQ ID NO:33)

Figure 26 AD

1			GAAGCCAATA CTTCGGTTAT	
51		 	TGGGAACGGG ACCCTTGCCC	
101			GTGTGGCGGA CACACCGCCT	
151			GTGTGCGCCG CACACGCGGC	
201	GAAGTGACAA CTTCACTGTT		GATGTTGTAG CTACAACATC	
251			GGGAAAACTG CCCTTTTGAC	
301			TAGCGCGTAA ATCGCGCATT	
351		 -	AGACTCGCCC TCTGAGCGGG	
401		 	TTGGCGTTTT AACCGCAAAA	
451			CATATCATAA GTATAGTATT	
501			TGTTGACATT ACAACTGTAA	
551	•	 	ATTAGTTCAT TAATCAAGTA	
601			ATGGCCCGCC TACCGGGCGG	
651			ATGACGTATG TACTGCATAC	
701	AACGCCAATA TTGCGGTTAT			TATTTACGGT ATAAATGCCA
751				AAGTACGCCC TTCATGCGGG
801				ATGCCCAGTA TACGGGTCAT

Figure 27A

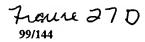
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951		ACTCACGGGG TGAGTGCCCC			
1001		TTTTGGCACC AAAACCGTGG			
1051		CCCATTGACG GGGTAACTGC			
1101	CAGATATATT	GCAGAGCTCG CGTCTCGAGC	AAATCACTTG	GCAGTCTAGC	GGACCTCTGC
1151		TGTTTTGACC ACAAAACTGG			
1201	AGGCGCCGGC	CCTTGCCACG	TAACCTTGCG	CCTAAGGGGC	
1251	CTCTAGACGG	ACCATGGCCG TGGTACCGGC	CGTTCACCAG	GTTCTCCAGG	CACGGGCCGA
1301	CCAGGTGGCA	GAGGGAGAGG CTCCCTCTCC	TACTCCTCCC	GGCTCGGGCG	GCGGCTGTCC
1351	CACTCCTCCT	CCGAGCCCGC GGCTCGGGCG	GCGTCACCCG	CACCCGCGGC	ACAGGTCCCT
1401	••••	CACGGCGCCA GTGCCGCGGT			
1451	GGCTGACGCG	CTGGCTGGAG GACCGACCTC	CGGGTCCTCC	TGCTCCTCCA	CCCGAAGGGG
1501	CACTCCGGGG	AGGTGCCCCT TCCACGGGGA	CTCCGGGTAC	TGGATGTTCC	CGCGGCACCT
1551	GGACAGGGTG	TTCCTGAAGG AAGGACTTCC	TCTTCCCGCC	GGACCTCCCG	GACTAGGTGA
		CGTCCTGTAG	GACCTGGACA	CCCACATGGT	GTGGGTCCCG
		TGACCGTCTT	GATGTGGGGG	CCGGGGCCGT	AGTCCAAGGG
		CCGACCACGA	AGTTCGACCA	CGGGCACCTC	GGGCTCTTCC
1751	TGGAGGAGGC ACCTCCTCCG				CCCCATGTCC GGGGTACAGG

Figure 27B

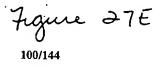
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	TGTTCCTGAC	GATTTCGGGC	CCGTCTAGAC	GACACGGAAG	ATCAACGGTC
1951	CCATCTGTTG	TTTGCCCCTC	CCCCGTGCCT	TCCTTGACCC	TGGAAGGTGC
	GGTAGACAAC	AAACGGGGAG	GGGGCACGGA	AGGAACTGGG	ACCTTCCACG
2001	CACTCCCACT	GTCCTTTCCT	AATAAAATGA	GGAAATTGCA	TCGCATTGTC
	GTGAGGGTGA	CAGGAAAGGA	TTATTTTACT	CCTTTAACGT	AGCGTAACAG
2051	TGAGTAGGTG	TCATTCTATT	CTGGGGGGTG	GGGTGGGGCA	GGACAGCAAG
	ACTCATCCAC	AGTAAGATAA	GACCCCCCAC	CCCACCCCGT	CCTGTCGTTC
			•		
2101	GGGGAGGATT	GGGAAGACAA	TAGCAGGCAT	GCTGGGGATG	CGGTGGGCTC
	CCCCTCCTAA	CCCTTCTGTT	ATCGTCCGTA	CGACCCCTAC	GCCACCGAG
2151	TATGGCCGAT	CGGCGCGCCG	TACTGAAATG	TGTGGGCGTG	GCTTAAGGGT
	ATACCGGCTA	GCCGCGCGGC	ATGACTTTAC	ACACCCGCAC	CGAATTCCCA
2201	GGGAAAGAAT	ATATAAGGTG	GGGGTCTTAT	GTAGTTTTGT	ATCTGTTTTG
	CCCTTTCTTA	TATATTCCAC	CCCCAGAATA	CATCAAAACA	TAGACAAAAC
2251	CAGCAGCCGC	CGCCGCCATG	AGCACCAACT	CGTTTGATGG	AAGCATTGTG
	GTCGTCGGCG	GCGGCGGTAC	TCGTGGTTGA	GCAAACTACC	TTCGTAACAC
				•	
2301	AGCTCATATT	TGACAACGCG	CATGCCCCCA	TGGGCCGGGG	TGCGTCAGAA
	TCGAGTATAA	ACTGTTGCGC	GTACGGGGGT	ACCCGGCCCC	ACGCAGTCTT
	· = · · ·				
2351	TGTGATGGGC	TCCAGCATTG	ATGGTCGCCC	CGTCCTGCCC	GCAAACTCTA
	ACACTACCCG	AGGTCGTAAC	TACCAGCGGG	GCAGGACGGG	CGTTTGAGAT
				•	
2401	CTACCTTGAC	CTACGAGACC	GTGTCTGGAA	CGCCGTTGGA	GACTGCAGCC
	GATGGAACTG	GATGCTCTGG	CACAGACCTT	GCGGCAACCT	CTGACGTCGG
			•		
2451	TCCGCCGCCG	CTTCAGCCGC	TGCAGCCACC	GCCCGCGGGA	TTGTGACTGA
	AGGCGGCGGC	GAAGTCGGCG	ACGTCGGTGG	CGGGCGCCCT	AACACTGACT
2501	CTTTGCTTTC	CTGAGCCCGC	TTGCAAACAĠ	TGCAGCTTCC	CGTTCATCCG
	GAAACGAAAG	GACTCGGGCG	AACGTTTGTC	ACGTCGAAGG	GCAAGTAGGC
2551	CCCGCGATGA	CAAGTTGACG	GCTCTTTTGG	CACAATTGGA	TTCTTTGACC
	GGGCGCTACT	GTTCAACTGC	CGAGAAAACC	GTGTTAACCT	AAGAAACTGG
		•			
2601	CGGGAACTTA	ATGTCGTTTC	TCAGCAGCTG	TTGGATCTGC	GCCAGCAGGT
	GCCCTTGAAT	TACAGCAAAG	AGTCGTCGAC	AACCTAGACG	CGGTCGTCCA
2651	TTCTGCCCTG	AAGGCTTCCT	CCCCTCCCAA	TGCGGTTTAA	AACATAAATA
	AAGACGGGAC	TTCCGAAGGA	GGGGAGGGTT	ACGCCAAATT	TTGTATTTAT
2701	AAAAACCAGA	CTCTGTTTGG	ATTTGGATCA	AGCAAGTGTC	TTGCTGTCTT
	TTTTTGGTCT	GAGACAAACC	TAAACCTAGT	TCGTTCACAG	AACGACAGAA

Figure 27C

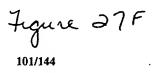
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		AAAACGCGCG			
2801		CTGTGTATTT GACACATAAA			
2851		CATGGGCATA GTACCCGTAT			
2001		CATGCTGCGG			
2901	ACGTCTCGAA	GTACGACGCC	CCACCACAAC	ATCTACTAGG	TCAGCATCGT
2951		GCGTGGTGCC CGCACCACGG			
3001		GCCCTTGGTG			
3001	GGTCCCCGTC	CGGGAACCAC	ATTCACAAAT	GTTTCGCCAA	TTCGACCCTA
3051		GTGGGGATAT			
		CACCCTATA			
3101	GGCTATGTTC	CCAGCCATAT GGTCGGTATA	CCCTCCGGGG	ATTCATGTTG TAAGTACAAC	TGCAGAACCA ACGTCTTGGT
				•	
3151		GTATCCGGTG CATAGGCCAC			
3201	CCAAATCCGT	GGAAGAACTT	GGAGACGCCC	TTGTGACCTC	CAAGATTTTC
3201		CCTTCTTGAA			
3251		TCCATAATGA			
		AGGTATTACT	•		
3301		TCTGGGATCA			
		AGACCCTAGT			
3351		CCATTTTTAC			
	AGCAGTATCC	GGTAAAAATG	TTTCGCGCCC	GCCTCCCACG	GTCTGACGCC
3401					CAGATTTGCA
	ATATTACCAA	GGTAGGCCGG	GTCCCCGCAT	CAATGGGAGT	GTCTAAACGT
3451	TTTCCCACGC	TTTGAGTTCA	GATGGGGGGA	TCATGTCTAC	CTGCGGGGCG
	AAAGGGTGCG	AAACTCAAGT	CTACCCCCCT	AGTACAGATG	GACGCCCCGC
3501	ATGAAGAAAA TACTTCTTTT	CGGTTTCCGG GCCAAAGGCC	GGTAGGGGAG CCATCCCCTC	ATCAGCTGGG TAGTCGACCC	AAGAAAGCAG TTCTTTCGTC
					TAAATCACAC
3551	CAAGGACTCG	TCGACGCTGA	ATGGCGTCGG	CCACCCGGGC	ATTTAGTGTG
3601	CTATTACCGG	CTGCAACTGG	TAGTTAAGAG	AGCTGCAGCT	GCCGTCATCC
	GATAATGGCC	GACGTTGACC	ATCAATTCTC	TCGACGTCGA	CGGCAGTAGG
3651	CTGAGCAGGG	GGGCCACTTC	GTTAAGCATG	TCCCTGACTC	GCATGTTTTC
	GACTCGTCCC	CCCGGTGAAG	CAATTCGTAC	AGGGACTGAG	CGTACAAAAG



3701		TCCGCCAGAA AGGCGGTCTT			
3751		AAAGTTTTTC TTTCAAAAAG			
3801		TTTGACCAAG AAACTGGTTC			
3851	_	GCATCTCGAT CGTAGAGCTA			
3901		CTGTACGGCA GACATGCCGT			
3951		CCACGGGCGC GGTGCCCGCG			
4001		GCGCTCCGGG CGCGAGGCCC			
4051	••••	GTGCTGAAGC CACGACTTCG			
4101		GACCATGGTG CTGGTACCAC			
4151		GCTTGCCCTT CGAACGGGAA			
4201		GCGTAGAGCT CGCATCTCGA			
4251		GCCGCAGGCC			CACGAGCCAG. GTGCTCGGTC
4301		GCCGTTCGGG CGGCAAGCCC			
4351		TTACCTCTGG AATGGAGACC			
4401	CGAAAAGGCT GCTTTTCCGA				CCTGTCCTCG GGACAGGAGC
4451	AGCGGTGTTC TCGCCACAAG				ACTCTGAGAC TGAGACTCTG
		CAGGTCCGGT	CGTGCTTCCT	CCGATTCACC	CTCCCCATCG
4551	GGTCGTTGTC CCAGCAACAG				AAGACACATG TTCTGTGTAC
4601			•		TGTAGGCCAC ACATCCGGTG



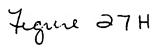
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	•			GCTCCCGGTC	
4751	GAGTACTCCC	TCTGAAAAGC	GGGCATGACT	TCTGCGCTAA	GATTGTCAGT
				AGACGCGATT	
4801				CTGGCCCGCG	
				GACCGGGCGC	
4851				AGACAATCTT	
				TCTGTTAGAA	
4901				TTGGACAGCA	
				AACCTGTCGT	
4951				GGCGCGCTCC	
				CCGCGCGAGG	
5001				ACCGCCATTC	
				TGGCGGTAAG	
5051				CGCCAACCGC	
				GCGGTTGGCG	
5101				TCCGCGTAGG	
				AGGCGCATCC	
5151				AGAATGGCGG	
				TCTTACCGCC	
5201	AGCTGCGTCT	CGTCCGGGG	GTCTGCGTCC	ACGGTAAAGA	CCCCGGGCAG
				TGCCATTTCT TCCTTGCAAG	
5251				AGGAACGTTC	
5201			-	CGTATGGGTT	
5301				GCATACCCAA	
5351				GCGTACATGC CGCATGTACG	
F401					GGGTAGCATC
5401					CCCATCGTAG
5451					CTGCGAGGGA CACGCTCCCT
5501					CTGCTCGGAA
					GACGAGCCTT
5551					GTTGGACGCT
	CTGATAGACG	GACTTCTACC	GTACACTCAA	CCTACTATAC	CAACCTGCGA



5651	GAGGCGTAGG CTCCGCATCC		AGCTCGGCGG TCGAGCCGCC	
5701		 	GATGATGTCA CTACTACAGT	
5751			GGACAAACTC CCTGTTTGAG	
5801			GCCTCCGAAC CGGAGGCTTG	
5851		 	GGCGCAGCAT CCGCGTCGTA	
5901			GGAGCGAGGT CCTCGCTCCA	
5951	-		TACTGGTATT ATGACCATAA	
6001		 	AAAGTCCGTG TTTCAGGCAC	
6051		 	CGTTGAAGAG GCAACTTCTC	
6101		 	AAGGGTCCCG TTCCCAGGGC	
6151		-	GATCTCGTCA CTAGAGCAGT	
6201		 	AGCGCGGGAT TCGCGCCCTA	
6251	4.4.40	 	AGCTCTTCAG TCGAGAAGTC	
6301		 	ATGAGGGTTG TACTCCCAAC	
6351				GTCGCGAAAG CAGCGCTTTC
6401	GTCCTAAACT CAGGATTTGA		TCTGGGGTGA AGACCCCACT	
6451				GCGGCTAGGT CGCCGATCCA
6501				CATGACCAGC GTACTGGTCG

Figure 27G

6601		GTGACAAAGA CACTGTTTCT		
6651		GATCTCCCGC CTAGAGGGCG		
6701		AGTCCCTGCG TCAGGGACGC		
6751		CAGTACTGGC GTCATGACCG		
6801	GGTTGACCTG CCAACTGGAC	ACGACCGCGC TGCTGGCGCG		
6851		GGTTTGGCTG CCAAACCGAC	 	
6901		TGCTCGAGGG ACGAGCTCCC		
6951		AGTCCAGATG TCAGGTCTAC	 	
7001		GATGGGAGCT CTACCCTCGA		
7051	•••	AGCTCCTGCA TCGAGGACGT		
7101		CAGGTGATAC GTCCACTATG		
7151		GCAAGAGGCC CGTTCTCCGG	 	
7201		TGGGCCGCGG ACCCGGCGCCC		
7251		CGAGCCCCCG GCTCGGGGGC		
7301	GAGGGGGCAG CTCCCCCGTC			CTGGTGCTGC GACCACGACG
7351	GCGCGTAGGT CGCGCATCCA			TCTCCTGAAT AGAGGACTTA
7401	CTGGCGCCTC GACCGCGGAG	TGCGTGAAGA ACGCACTTCT		
7451	AGAGTTCGAC TCTCAAGCTG			CTGGCGCAAA GACCGCGTTT



7551				GCGTCCGGCT CGCAGGCCGA	
7601				TGAGCTGCGA ACTCGACGCT	
7651				ACCACGCCCC TGGTGCGGGG	
7701				GAGCTCCACG CTCGAGGTGC	
7751				GGTAGTTGAG CCATCAACTC	
7801				CAGCGTCGCA GTCGCAGCGT	
7851				CATGGCCTCG GTACCGGAGC	
7901		••••		CCGACACGGT GGCTGTGCCA	
7951				TCGCGCACCT AGCGCGTGGA	
8001				CTCCTCTTCC GAGGAGAAGG	
8051				GAGGGGGGAC CTCCCCCTG	
8101				CGCTCGATCA GCGAGCTAGT	
8151				GCCGTTCTCG CGGCAAGAGC	CGGGGGCGCA GCCCCCGCGT
8201				TATGGGTTGG ATACCCAACC	CGGGGGGCTG GCCCCCGAC
8251					ATTGTTGTGT TAACAACACA
8301	AGGTACTCCG TCCATGAGGC	CCGCCGAGGG GGCGGCTCCC	ACCTGAGCGA TGGACTCGCT	GTCCGCATCG CAGGCGTAGC	ACCGGATCGG TGGCCTAGCC
8351					AGGTAGGCTG TCCATCCGAC
8401					TTCTGGCGGA AAGACCGCCT

Figure 27I

8501				CCTGCTGAAT GGACGACTTA	
8551				CGGCGCAGGT GCCGCGTCCA	
8601	GTCTTGCATG CAGAACGTAC	AGCCTTTCTA TCGGAAAGAT	CCGGCACTTC GGCCGTGAAG	TTCTTCTCCT AAGAAGAGGA	TCCTCTTGTC AGGAGAACAG
8651	CTGCATCTCT GACGTAGAGA	TGCATCTATC ACGTAGATAG	GCTGCGGCGG CGACGCCGCC	CGGCGGAGTT GCCGCCTCAA	TGGCCGTAGG ACCGGCATCC
8701	TGGCGCCCTC ACCGCGGGAG	TTCCTCCCAT AAGGAGGGTA	GCGTGTGACC CGCACACTGG	CCGAAGCCCC GGCTTCGGGG	TCATCGGCTG AGTAGCCGAC
8751				GGCTAATATG CCGATTATAC	
8801				TGTCCACAAA ACAGGTGTTT	
8851	GCGCCCGTGT CGCGGGCACA	TGATGGTGTA ACTACCACAT	AGTGCAGTTG TCACGTCAAC	GCCATAACGG CGGTATTGCC	ACCAGTTAAC TGGTCAATTG
8901	GGTCTGGTGA CCAGACCACT	CCCGGCTGCG	AGAGCTCGGT TCTCGAGCCA	GTACCTGAGA CATGGACTCT	CGCGAGTAAG GCGCTCATTC
8951				TCCGCACCAG AGGCGTGGTC	
9001				TAGAGGGGCC ATCTCCCCGG	AGCGTAGGGT . TCGCATCCCA
9051	GGCCGGGGCT CCGGCCCCGA	CCGGGGGCGA	GATCTTCCAA CTAGAAGGTT	CATAAGGCGA GTATTCCGCT	TGATATCCGT ACTATAGGCA
9101	AGATGTACCT TCTACATGGA	GGACATCCAG CCTGTAGGTC	GTGATGCCGG CACTACGGCC	CGCCGCCACCA	GGAGGCGCGC CCTCCGCGCG
9151	GGAAAGTCGC CCTTTCAGCG	GGACGCGGTT	CCAGATGTTG GGTCTACAAC	CGCAGCGGCA GCGTCGCCGT	AAAAGTGCTC TTTTCACGAG
9201	CATGGTCGGG GTACCAGCCC	ACGCTCTGGC TGCGAGACCG	CGGTCAGGCG	CGCGCAATCG GCGCGTTAGC	TTGACGCTCT AACTGCGAGA
9251	AGACCGTGCA TCTGGCACGT	AAAGGAGAGC TTTCCTCTCG	CTGTAAGCGG GACATTCGCC	GCACTCTTCC	GTGGTCTGGT CACCAGACCA
9301	GGATAAATTC CCTATTTAAG	GCAAGGGTAT GCTTCCCATA	CATGGCGGAC	GACCGGGGTT	CGAGCCCCGT GCTCGGGGCA
9351	ATCCGGCCGT TAGGCCGGCA	CCGCCGTGAT GGCGGCACTA	CCATGCGGTT GGTACGCCAA	ACCGCCCGCG TGGCGGGCGC	TGTCGAACCC ACAGCTTGGG

Figure 27J

9451	ccccccccc			GCCACTGGCC CGGTGACCGG	
9501	TAAGCGGTTA ATTCGCCAAT			AAGTGGCTCG TTCACCGAGC	
9551				CGGGACCCCC GCCCTGGGGG	
9601				TTTGCCTCCC AAACGGAGGG	
9651	GACCCCGCTT CTGGGGCGAA			GGACGAGCCC CCTGCTCGGG	
9701	TTTCCCAGAT AAAGGGTCTA			TGCGCCCCCC ACGCGGGGGGG	
9751				AGGGCACCCT TCCCGTGGGA	
9801				TGACGCGGCA ACTGCGCCGT	
9851	ATTACGAACC TAATGCTTGG		• •	ACTACCTGGA TGATGGACCT	
9901				TCTCCTGAGC AGAGGACTCG	
9951		•		GTACGTGCCG CATGCACGGC	
10001				AGGAGATGCG TCCTCTACGC	
10051				CTGAATCGCG GACTTAGCGC	
10101				AACCGGGATT TTGGCCCTAA	
10151	GCGCACACGT CGCGTGTGCA			CCGCATACGA GGCGTATGCT	
10201	AACCAGGAGA TTGGTCCTCT			AACAACCACG TTGTTGGTGC	
10251	TGTGGCGCGC ACACCGCGCG			GATGCATCTG CTACGTAGAC	
10301	TAAGCGCGCT ATTCGCGCGA			AGCCGCTCAT TCGGCGAGTA	

Figure. 27 K

10401	GCTAAACATA	GTAGAGCCCG	AGGGCCGCTG	GCTGCTCGAT	TTGATAAACA
			TCCCGGCGAC		
10451	TCCTGCAGAG	CATAGTGGTG	CAGGAGCGCA	GCTTGAGCCT	GGCTGACAAG
			GTCCTCGCGT		
10501			CATGCTTAGC		
			GTACGAATCG		
10551			ACGTTCCCAT		
			TGCAAGGGTA		
10601			GCGCTGAAGG		
			CGCGACTTCC		
10651	CTGGGCGTTT	ATCGCAACGA	GCGCATCCAC CGCGTAGGTG	AAGGCCGTGA	CCCACTCCCC
10701	GCGGCGCGAG	CTCAGCGACC	GCGAGCTGAT CGCTCGACTA	GCACAGCCTG	CAAAGGGCCC
	CGCCGCGCTC	GAGTCGCTGG	CGCTCGACTA	CGIGICGGAC	GIIICCCGGG
10751			GATAGAGAGG		
			CTATCTCTCC		
10801	GGCGCTGACC	TGCGCTGGGC	CCCAAGCCGA	CGCGCCCTGG	AGGCAGCTGG
			GGGTTCGGCT		
10851	GGCCGGACCT	GGGCTGGCGG	TGGCACCCGC	GCGCGCTGGC	AACGTCGGCG
			ACCGTGGGCG		
10901	GCGTGGAGGA	ATATGACGAG	GACGATGAGT	ACGAGCCAGA	GGACGGCGAG
			CTGCTACTCA		
10951			GATCAGATGA CTAGTCTACT		
		•			
11001	GCGGTGCGGG	CGGCGCTGCA	CTCGGTCGGC	ACCCCCAAT	ACTCCACGGA
	CGCCACGCCC	GCCGCGACGT	CICGGICGGC	AGGCCGGAAI	1949919001
11051	CGACTGGCGC	CAGGTCATGG	ACCGCATCAT	GTCGCTGACT	GCGCGCAATC
			TGGCGTAGTA		
11101	CTGACGCGTT	CCGGCAGCAG	CCGCAGGCCA	ACCGGCTCTC	CGCAATTCTG
					GCGTTAAGAC
11151	GAAGCGGTGG	TCCCGGCGCG	CGCAAACCCC	ACGCACGAGA	AGGTGCTGGC
					TCCACGACCG
11201	GATCGTAAAC	GCGCTGGCCG	AAAACAGGGC	CATCCGGCCC	GACGAGGCCG
					CTGCTCCGGC
11251	GCCTGGTCTA	CGACGCGCTG	CTTCAGCGCG	TGGCTCGTTA	CAACAGCGGC
	CGGACCAGAT	GCTGCGCGAC	GAAGTCGCGC	ACCGAGCAAT	GTTGTCGCCG

Figure 27L

11351			CAACCTGGGC GTTGGACCCG	
11401			CCAACGTGCC GGTTGCACGG	
11451		 	CGGCTAATGG GCCGATTACC	
11501			AGACTATTTT TCTGATAAAA	
11551			GCCAGGCTTT CGGTCCGAAA	
11601		 	GGCGACCGCG CCGCTGGCGC	
11.651		- · ·	GCTGCTGCTA CGACGACGAT	
11701			CATACCTAGG GTATGGATCC	
11751			CATGTGGACG GTACACCTGC	
11801			GGGGCAGGAG CCCCGTCCTC	
11851		 	CCAACCGGCG GGTTGGCCGC	
11901		 	GAGCGCATTT CTCGCGTAAA	
11951			CGACGGGGTA GCTGCCCCAT	
12001			AACCGGGCAT TTGGCCCGTA	
12051	AACCGGCCGT TTGGCCGGCA		TACTTGCATC ATGAACGTAG	
	CGTGAACCCC GCACTTGGGG	 		
12151	CGCCCCTGG GCGGGGGACC			
12201	GGATTCCTCT CCTAAGGAGA	 	GTGTTTTCCC CACAAAAGGG	

Figure 27 M

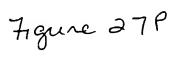
12301		-		CCGATCTAGG GGCTAGATCC	CGCTGCGGCC CGC
12351	••••			AGCTTGATAG TCGAACTATC	
12401				GGGCGAGGAG CCCGCTCCTC	
12451				AAAACCTGCC TTTTGGACGG	
12501			-	AAGATGAGTA TTCTACTCAT	
12551				ececececec cccececcce	
12601				TGTGGGAGGA ACACCCTCCT	
12651				GGGAGTGGCA CCCTCACCGT	
12701				AAAAAAAAA TTTTTTTAA	
12751				GCACCGAGCG CGTGGCTCGC	
12801				ATGTATGAGG TACATACTCC	
12851				GCCAGTGGCG CGGTCACCGC	
12901				CGTTTGTGCC GCAAACACGG	
,12951				CGTTACTCTG GCAATGAGAC	
13001	CCTATTCGAC GGATAAGCTG			GGACAACAAG CCTGTTGTTC	
13051	TGGCATCCCT ACCGTAGGGA				GACCACGGTC CTGGTGCCAG
13101	ATTCAAAACA TAAGTTTTGT				AGACCATCAA TCTGGTAGTT
13151					ATCCTGCATA TAGGACGTAT

Figure 27N

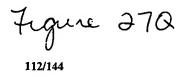
13251		TGTCGCGCTT ACAGCGCGAA			
13301		GTGGAGTTCA CACCTCAAGT			
13351		CCTTATGAAC GGAATACTTG			
13401	GGCAGACAGA	ACGGGGTTCT TGCCCCAAGA	GGAAAGCGAC	ATCGGGGTAA	AGTTTGACAC
13451	CCGCAACTTC	AGACTGGGGT	TTGACCCCGT	CACTGGTCTT	GTCATGCCTG
13501	GGGTATATAC	AAACGAAGCC	TTCCATCCAG	ACATCATTTT	GCTGCCAGGA
13551		TTTGCTTCGG ACTTCACCCA	•		
13601		TGAAGTGGGT CCCTTCCAGG			
	GTTCGCCGTT	GGGAAGGTCC	TCCCGAAATC	CTAGTGGATG	CTACTAGACC
13651		CATTCCCGCA GTAAGGGCGT			
13701		ACACCGAACA TGTGGCTTGT			
13751		GGCGCGGAAG @CGCGCCTTC			
13801		GGACATGAAC CCTGTACTTG			
13851		AGGAGAAGCG TCCTCTTCGC		•	
13901		GCGCAACCCG CGCGTTGGGC			
13951	TCAAACCCCT AGTTTGGGGA	GACAGAGGAC CTGTCTCCTG			
14001	AATGACAGCA TTACTGTCGT	CCTTCACCCA GGAAGTGGGT			
14051	CGGCGACCCT	CAGACCGGAA GTCTGGCCTT			
14101	ACGTAACCTG		CAGGTCTACT	GGTCGTTGCC	AGACATGATG

Tigure 270

14201		GAGCTGTTGC CTCGACAACG			
14251		CTCCCAACTC GAGGGTTGAG			
14301		TTCCCGAGAA AAGGGCTCTT	· · · - · · •		
14351		GTCAGTGAAA CAGTCACTTT			
14401		CAACAGCATC GTTGTCGTAG			
14451		GCACCTGCCC CGTGGACGGG			
14501		CTATCGAGCC GATAGCTCGG			
14551		CAATAACACA GTTATTGTGT			
14601		CCAAGAAGCG GGTTCTTCGC			
14651		GCGCCCTGGG CGCGGGACCC			
14701		TGACGCCATC ACTGCGGTAG			
14751		CGCCACCAGT GCGGTGGTCA			
14801	-	GCCCGGCGCT CGGGCCGCGA		-	•
14851		CCACCGCCGC GCTGGCGGCG			
14901	GCGGCCCTGC CGCCGGGACG	TTAACCGCGC AATTGGCGCG			
14951	GGCCGCTCGA CCGGCGAGCT	AGGCTGGCCG TCCGACCGGC			
15001	GGCGACGAGC CCGCTGCTCG	GGCCGCCGCA CCGGCGCGT			
15051	GGTCGCAGGG CCAGCGTCCC	GCAACGTGTA CGTTGCACAT			



15151				CGGCGGCGGC	
	TGAATCTGAG	CATGACAACA	TACATAGGTC	GCCGCCGCCG	CGCGTTGCTT
15201				ATGCTCCAGG	
				TACGAGGTCC	
15251	GGAGATCTAT				
	CCTCTAGATA	CCGGGGGGCT	TCTTCCTTCT	CGTCCTAATG	TTCGGGGCTT
15301				ATGATGATGA	
	TCGATTTCGC	CCAGTTTTTC	TTTTTCTTTC	TACTACTACT	ACTTGAACTG
15351	•			CCCAGGCGAC	
	CTGCTCCACC	TTGACGACGT	GCGATGGCGC	GGGTCCGCTG	CCCATGTCAC
15401		•		ACCCGGCACC	
	CTTTCCAGCT	GCGCATTTTG	CACAAAACGC	TGGGCCGTGG	TGGCATCAGA
15451				ACAAGCGCGT	
				TGTTCĞCGCA	
15501				GCCAACGAGC	
	CACATGCCGC	TGCTCCTGGA	CGAACTCGTC	CGGTTGCTCG	CGGAGCCCCT
15551		•		GCTGGCGTTG	
·	CAAACGGATG	CCTTTCGCCG	TATTCCTGTA	CGACCGCAAC	GGCGACCTGC
15601				TAACACTGCA	
	TCCCGTTGGG	TTGTGGATCG	GATTTCGGGC	ATTGTGACGT	CGTCCACGAC
15651			· · · · · · · · · · · · · · · · · · ·	GGCCTAAAGC	
	GGGCGCGAAC	GTGGCAGGCT	TCTTTTCGCG	CCGGATTTCG	CGCTCAGACC
15701				ACCCAAGCGC	-
	ACTGAACCGT	GGGTGGCACG	TCGACTACCA	TGGGTTCGCG	GTCGCTGACC
15751				CTGGGCTGGA	
	TTCTACAGAA	CCTTTTTTAC	TGGCACCTTG	GACCCGACCT	CGGGCTCCAG
15801				GGACTGGGCG	
	GCGCACGCCG	GTTAGTTCGT	CCACCGCGGC	CCTGACCCGC	ACGTCTGGCA
15851	GGACGTTCAG				
				GTCATAACGG	
15901	AGGGCATGGA				
	TCCCGTACCT	CTGTGTTTGC	AGGGGCCAAC	GGAGTCGCCA	CCGCCTACGG
15951	GCGGTGCAGG				
	CGCCACGTCC	GCCAGCGACG	CCGGCGCAGG	TTCTGGAGAT	GCCTCCACGT
16001					CCGCGCCGTT
	TTGCCTGGGC	ACCTACAAAG	CGCAAAGTCG	GGGGGCCGCG	GGCGCGGCAA



16051				TGCCCGAATA ACGGGCTTAT	
16101	-			GGCTACACCT CCGATGTGGA	
16151				CACTGGAACC GTGACCTTGG	
16201				TTTCCGTGCG AAAGGCACGC	
16251				ACAGCGCGCT TGTCGCGCGA	
16301				TGCAGATATG ACGTCTATAC	
16351			•	GAGGAAGAAT CTCCTTCTTA	
16401				GGCATGCGTC CCGTACGCAG	
16451				GCGCGGCGGT CGCGCCGCCA	
16501				GCGCCGTGCC CGCGGCACGG	
16551				TTAAAAACAA AATTTTTGTT	
16601	-			ACGCTCGCTT TGCGAGCGAA	
16651				GCGTCTCTGG CGCAGAGACC	
16701				AGATATCGGC TCTATAGCCG	
16751	TGAGCGGTGG ACTCGCCACC	CGCCTTCAGC GCGGAAGTCG	TGGGGCTCGC ACCCCGAGCG	TGTGGAGCGG ACACCTCGCC	CATTAAAAAT GTAATTTTTA
16801	TTCGGTTCCA AAGCCAAGGT			AAGGCCTGGA TTCCGGACCT	
16851	AGGCCAGATG TCCGGTCTAC	CTGAGGGATA GACTCCCTAT	AGTTGAAAGA TCAACTTTCT	GCAAAATTTC CGTTTTAAAG	CAACAAAAGG GTTGTTTTCC
16901	TGGTAGATGG ACCATCTACC				
16951	CAGGCAGTGC GTCCGTCACG				GCCCTCCCGT CGGGAGGGCA



17051	AAAAGCGTCC TTTTCGCAGG		CTCTGGTGAC GAGACCACTG	
17161		 	CAAGGCCTGC GTTCCGGACG	
17151			GGGCCAGCAC CCCGGTCGTG	-
17201		 	AGCAGAAACC TCGTCTTTGG	
17251		· · · · · · · · · · · · · · · · · · ·	AGCCGCGCGT TCGGCGCGCA	
17301			CGTAGCCAGT GCATCGGTCA	
17351		 	GGGTGCAATC CCCACGTTAG	
17401		 	ATGTGTGTCA TACACACAGT	
17451		 	GCGCGCGCCC	
17501		 	TCTTACATGC AGAATGTACG	
17551		 	GCTGGTGCAG CGACCACGTC	
17601		 	AGTTTAGAAA TCAAATCTTT	
17651			TCCCAGCGTT AGGGTCGCAA	
17701			GTACTCGTAC CATGAGCATG	
17751	TCACCCTAGC AGTGGGATCG		TGGACATGGC ACCTGTACCG	
17801	TTTGACATCC AAACTGTAGG		CCTACTTTTA GGATGAAAAT	
17851	TGGCACTGCC ACCGTGACGG		GGGTGCCCCA CCCACGGGGT	
17901	AATGGGATGA TTACCCTACT		TAAACCTAGA ATTTGGATCT	

Figure 275

17951	GATGACAACG CTACTGTTGC	AAGACGAAGT TTCTGCTTCA	AGACGAGCAA TCTGCTCGTT	GCTGAGCAGC CGACTCGTCG	AAAAAACTCA TTTTTTGAGT
18001				AAATATTACA TTTATAATGT	
18051				AATATGCCGA TTATACGGCT	
18101				TGGTACGAAA ACCATGCTTT	
18151				TACCCCAATG ATGGGGTTAC	
18201	ACGGTTCATA TGCCAAGTAT	TGCAAAACCC ACGTTTTGGG	ACAAATGAAA TGTTTACTTT	ATGGAGGGCA TACCTCCCGT	AGGCATTCTT TCCGTAAGAA
18251				CAAGTGGAAA GTTCACCTTT	
18301	CTCAACTACT GAGTTGATGA	GAGGCAGCCG CTCCGTCGGC	CAGGCAATGG GTCCGTTACC	TGATAACTTG ACTATTGAAC	ACTCCTAAAG TGAGGATTTC
18351				AAACCCCAGA TTTGGGGTCT	
18401				TCACGAGAAC AGTGCTCTTG	
18451	ACAATCTATG TGTTAGATAC	CCCAACAGGC GGGTTGTCCG	CTAATTACAT GATTAATGTA	TGCTTTTAGG ACGAAAATCC	GACAATTTTA CTGTTAAAAT
18501				ATATGGGTGT TATACCCACA	
18551				TTGCAAGACA AACGTTCTGT	GAAACACAGA CTTTGTGTCT
18601					ACCÁGGTACT TGGTCCATGA
18651	TTTCTATGTG AAAGATACAC	GAATCAGGCT CTTAGTCCGA	GTTGACAGCT CAACTGTCGA	ATGATCCAGA TACTAGGTCT	TGTTAGAATT ACAATCTTAA
18701	ATTGAAAATC TAACTTTTAG	ATGGAACTGA TACCTTGACT	AGATGAACTT TCTACTTGAA	CCAAATTACT GGTTTAATGA	GCTTTCCACT CGAAAGGTGA
18751	GGGAGGTGTG CCCTCCACAC	ATTAATACAG TAATTATGTC	AGACTCTTAC TCTGAGAATG	CAAGGTAAAA GTTCCATTTT	CCTAAAACAG GGATTTTGTC
18801	GTCAGGAAAA CAGTCCTTTT	TGGATGGGAA ACCTACCCTT	AAAGATGCTA TTTCTACGAT	CAGAATTTTC GTCTTAAAAG	AGATAAAAAT TCTATTTTA
18851	GAAATAAGAG CTTTATTCTC	TTGGAAATAA AACCTTTATT	TTTTGCCATG AAAACGGTAC	GAAATCAATC CTTTAGTTAG	TAAATGCCAA ATTTACGGTT

Figure 27T.

	•				
18951	AGCTAAAGTA	CAGTCCTTCC	AACGTAAAAA	TTTCTGATAA	CCCAAACACC
	TYCEATTTYAT	GTCAGGAAGG	ተ ብርር እ ተብጥጥጥ	AAAGACTATT	CCCTTTCTCC
					0001110190
19001		TGAACAAGCG			
	ATGCTGATGT	ACTTGTTCGC	TCACCACCGA	GGGCCCGATC	ACCTGACGAT
19051	САТТАВССТТ	GGAGCACGCT	GCTCCCTTCA	СТАТАТССАС	AACCTCAACC
13031					
	GTAATTGGAA	CCTCGTGCGA	LUAGGGAAUT	GATATACCTG	TIGCAGTIGG
				•	
19101	CATTTAACCA	CCACCGCAAT	GCTGGCCTGC	GCTACCGCTC	AATGTTGCTG
	GTAAATTGGT	GGTGGCGTTA	CGACCGGACG	CGATGGCGAG	TTACAACGAC
10151	0001180080	000000000	CDD0010100	a. camacama	
19151		GCTATGTGCC			
	CCGTTACCAG	CGATACACGG	GAAGGTGTAG	GTCCACGGAG	TCTTCAAGAA
19201	TGCCATTAAA	AACCTCCTTC	TCCTGCCGGG	CTCATACACC	TACGAGTGGA
		TTGGAGGAAG			
	ACGGIAAIII	1100noonno	MOGNEGGEEE	GAGIAIGIGG	NIGCICACCI
19251	ACTTCAGGAA	GGATGTTAAC	ATGGTTCTGC	AGAGCTCCCT	AGGAAATGAC
	TGAAGTCCTT	CCTACAATTG	TACCAAGACG	TCTCGAGGGA	TCCTTTACTG
				•	
19301	CON ACCOMPC	ACGGAGCCAG	ר א ישיים א ריישיים	C 3 T 3 C C 3 T T T T	CCCTTTACCC
19301			•		
	GATTCCCAAC	TGCCTCGGTC	GTAATICAAA	CTATCGTAAA	CGGAAATGCG
				•	
19351	CACCTTCTTC	CCCATGGCCC	ACAACACCGC	CTCCACGCTT	GAGGCCATGC
	GTGGAAGAAG	GGGTACCGGG	TGTTGTGGCG	CACCTCCCAA	CTCCGGTACG
	01001101110	0001110000	.0	Q.001.000.11	ciccooineo
19401		CACCAAÇGAC			
	AATCTTTGCT	GTGGTTGCTG	GTCAGGAAAT	TGCTGATAGA	GAGGCGGCGG
19451	ΑΑΓΑΨΩΟΨΟΤ	ACCCTATACC	CCCCAACCCT	ACCAACGTGC	CCATATCCAT
13331					
	TIGIACGAGA	TGGGATATGG	GCGGTTGCGA	TGGTTGCACG	GGTATAGGTA
19501	CCCCTCCCGC	AACTGGGCGG	CTTTCCGCGG	CTGGGCCTTC	ACGCGCCTTA
19501				-	
19501		AACTGGGCGG TTGACCCGCC		-	
	GGGGAGGCG	TTGACCCGCC	GAAAGGCGCC	GACCCGGAAG	TGCGCGGAAT
19501	GGGGAGGGCG AGACTAAGGA	TTGACCCGCC AACCCCATCA	GAAAGGCGCC CTGGGCTCGG	GACCCGGAAG GCTACGACCC	TGCGCGGAAT TTATTACACC
	GGGGAGGGCG AGACTAAGGA	TTGACCCGCC	GAAAGGCGCC CTGGGCTCGG	GACCCGGAAG GCTACGACCC	TGCGCGGAAT TTATTACACC
	GGGGAGGGCG AGACTAAGGA	TTGACCCGCC AACCCCATCA	GAAAGGCGCC CTGGGCTCGG	GACCCGGAAG GCTACGACCC	TGCGCGGAAT TTATTACACC
19551	GGGGAGGCG AGACTAAGGA TCTGATTCCT	TTGACCCGCC AACCCCATCA TTGGGGTAGT	GAAAGGCGCC CTGGGCTCGG GACCCGAGCC	GACCCGGAAG GCTACGACCC CGATGCTGGG	TGCGCGGAAT TTATTACACC AATAATGTGG
	GGGGAGGCG AGACTAAGGA TCTGATTCCT TACTCTGGCT	TTGACCCGCC AACCCCATCA TTGGGGTAGT CTATACCCTA	GAAAGGCGCC CTGGGCTCGG GACCCGAGCC CCTAGATGGA	GACCCGGAAG GCTACGACCC CGATGCTGGG ACCTTTTACC	TGCGCGGAAT TTATTACACC AATAATGTGG TCAACCACAC
19551	GGGGAGGCG AGACTAAGGA TCTGATTCCT TACTCTGGCT	TTGACCCGCC AACCCCATCA TTGGGGTAGT	GAAAGGCGCC CTGGGCTCGG GACCCGAGCC CCTAGATGGA	GACCCGGAAG GCTACGACCC CGATGCTGGG ACCTTTTACC	TGCGCGGAAT TTATTACACC AATAATGTGG TCAACCACAC
19551	GGGGAGGCG AGACTAAGGA TCTGATTCCT TACTCTGGCT ATGAGACCGA	TTGACCCGCC AACCCCATCA TTGGGGTAGT CTATACCCTA GATATGGGAT	GAAAGGCGCC CTGGGCTCGG GACCCGAGCC CCTAGATGGA GGATCTACCT	GACCCGGAAG GCTACGACCC CGATGCTGGG ACCTTTTACC TGGAAAATGG	TGCGCGGAAT TTATTACACC AATAATGTGG TCAACCACAC AGTTGGTGTG
19551	GGGGAGGCG AGACTAAGGA TCTGATTCCT TACTCTGGCT ATGAGACCGA CTTTAAGAAG	TTGACCCGCC AACCCCATCA TTGGGGTAGT CTATACCCTA GATATGGGAT GTGGCCATTA	GAAAGGCGCC CTGGGCTCGG GACCCGAGCC CCTAGATGGA GGATCTACCT CCTTTGACTC	GACCCGGAAG GCTACGACCC CGATGCTGGG ACCTTTTACC TGGAAAATGG TTCTGTCAGC	TGCGCGGAAT TTATTACACC AATAATGTGG TCAACCACAC AGTTGGTGTG TGGCCTGGCA
19551	GGGGAGGCG AGACTAAGGA TCTGATTCCT TACTCTGGCT ATGAGACCGA CTTTAAGAAG	TTGACCCGCC AACCCCATCA TTGGGGTAGT CTATACCCTA GATATGGGAT	GAAAGGCGCC CTGGGCTCGG GACCCGAGCC CCTAGATGGA GGATCTACCT CCTTTGACTC	GACCCGGAAG GCTACGACCC CGATGCTGGG ACCTTTTACC TGGAAAATGG TTCTGTCAGC	TGCGCGGAAT TTATTACACC AATAATGTGG TCAACCACAC AGTTGGTGTG TGGCCTGGCA
19551	GGGGAGGCG AGACTAAGGA TCTGATTCCT TACTCTGGCT ATGAGACCGA CTTTAAGAAG	TTGACCCGCC AACCCCATCA TTGGGGTAGT CTATACCCTA GATATGGGAT GTGGCCATTA	GAAAGGCGCC CTGGGCTCGG GACCCGAGCC CCTAGATGGA GGATCTACCT CCTTTGACTC	GACCCGGAAG GCTACGACCC CGATGCTGGG ACCTTTTACC TGGAAAATGG TTCTGTCAGC	TGCGCGGAAT TTATTACACC AATAATGTGG TCAACCACAC AGTTGGTGTG TGGCCTGGCA
19551 19601 19651	GGGGAGGCG AGACTAAGGA TCTGATTCCT TACTCTGGCT ATGAGACCGA CTTTAAGAAG GAAATTCTTC	TTGACCCGCC AACCCCATCA TTGGGGTAGT CTATACCCTA GATATGGGAT GTGGCCATTA CACCGGTAAT	GAAAGGCGCC CTGGGCTCGG GACCCGAGCC CCTAGATGGA GGATCTACCT CCTTTGACTC GGAAACTGAG	GACCCGGAAG GCTACGACCC CGATGCTGGG ACCTTTTACC TGGAAAATGG TTCTGTCAGC AAGACAGTCG	TGCGCGGAAT TTATTACACC AATAATGTGG TCAACCACAC AGTTGGTGTG TGGCCTGGCA ACCGGACCGT
19551 19601 19651	GGGGAGGCG AGACTAAGGA TCTGATTCCT TACTCTGGCT ATGAGACCGA CTTTAAGAAG GAAATTCTTC ATGACCGCCT	TTGACCCGCC AACCCCATCA TTGGGGTAGT CTATACCCTA GATATGGGAT GTGGCCATTA CACCGGTAAT GCTTACCCCC	GAAAGGCGCC CTGGGCTCGG GACCCGAGCC CCTAGATGGA GGATCTACCT CCTTTGACTC GGAAACTGAG AACGAGTTTG	GACCCGGAAG GCTACGACCC CGATGCTGGG ACCTTTTACC TGGAAAATGG TTCTGTCAGC AAGACAGTCG AAATTAAGCG	TGCGCGGAAT TTATTACACC AATAATGTGG TCAACCACAC AGTTGGTGTG TGGCCTGGCA ACCGGACCGT CTCAGTTGAC
19551 19601 19651	GGGGAGGCG AGACTAAGGA TCTGATTCCT TACTCTGGCT ATGAGACCGA CTTTAAGAAG GAAATTCTTC ATGACCGCCT	TTGACCCGCC AACCCCATCA TTGGGGTAGT CTATACCCTA GATATGGGAT GTGGCCATTA CACCGGTAAT	GAAAGGCGCC CTGGGCTCGG GACCCGAGCC CCTAGATGGA GGATCTACCT CCTTTGACTC GGAAACTGAG AACGAGTTTG	GACCCGGAAG GCTACGACCC CGATGCTGGG ACCTTTTACC TGGAAAATGG TTCTGTCAGC AAGACAGTCG AAATTAAGCG	TGCGCGGAAT TTATTACACC AATAATGTGG TCAACCACAC AGTTGGTGTG TGGCCTGGCA ACCGGACCGT CTCAGTTGAC
19551 19601 19651	GGGGAGGCG AGACTAAGGA TCTGATTCCT TACTCTGGCT ATGAGACCGA CTTTAAGAAG GAAATTCTTC ATGACCGCCT	TTGACCCGCC AACCCCATCA TTGGGGTAGT CTATACCCTA GATATGGGAT GTGGCCATTA CACCGGTAAT GCTTACCCCC	GAAAGGCGCC CTGGGCTCGG GACCCGAGCC CCTAGATGGA GGATCTACCT CCTTTGACTC GGAAACTGAG AACGAGTTTG	GACCCGGAAG GCTACGACCC CGATGCTGGG ACCTTTTACC TGGAAAATGG TTCTGTCAGC AAGACAGTCG AAATTAAGCG	TGCGCGGAAT TTATTACACC AATAATGTGG TCAACCACAC AGTTGGTGTG TGGCCTGGCA ACCGGACCGT CTCAGTTGAC
19551 19601 19651 19701	GGGGAGGCG AGACTAAGGA TCTGATTCCT TACTCTGGCT ATGAGACCGA CTTTAAGAAG GAAATTCTTC ATGACCGCCT TACTGGCGGA	TTGACCCGCC AACCCCATCA TTGGGGTAGT CTATACCCTA GATATGGGAT GTGGCCATTA CACCGGTAAT GCTTACCCCC	GAAAGGCGCC CTGGGCTCGG GACCCGAGCC CCTAGATGGA GGATCTACCT CCTTTGACTC GGAAACTGAG AACGAGTTTG TTGCTCAAAC	GACCCGGAAG GCTACGACCC CGATGCTGGG ACCTTTTACC TGGAAAATGG TTCTGTCAGC AAGACAGTCG AAATTAAGCG TTTAATTCGC	TGCGCGGAAT TTATTACACC AATAATGTGG TCAACCACAC AGTTGGTGTG TGGCCTGGCA ACCGGACCGT CTCAGTTGAC GAGTCAACTG
19551 19601 19651 19701	GGGGAGGCG AGACTAAGGA TCTGATTCCT TACTCTGGCT ATGAGACCGA CTTTAAGAAG GAAATTCTTC ATGACCGCCT TACTGGCGGA GGGGAGGGTT	TTGACCCGCC AACCCCATCA TTGGGGTAGT CTATACCCTA GATATGGGAT GTGGCCATTA CACCGGTAAT GCTTACCCC CGAATGGGGG ACAACGTTGC	GAAAGGCGCC CTGGGCTCGG GACCCGAGCC CCTAGATGGA GGATCTACCT CCTTTGACTC GGAAACTGAG AACGAGTTTG TTGCTCAAAC CCAGTGTAAC	GACCCGGAAG GCTACGACCC CGATGCTGGG ACCTTTTACC TGGAAAATGG TTCTGTCAGC AAGACAGTCG AAATTAAGCG TTTAATTCGC ATGACCAAAG	TGCGCGGAAT TTATTACACC AATAATGTGG TCAACCACAC AGTTGGTGTG TGGCCTGGCA ACCGGACCGT CTCAGTTGAC GAGTCAACTG ACTGGTTCCT
19551 19601 19651 19701	GGGGAGGCG AGACTAAGGA TCTGATTCCT TACTCTGGCT ATGAGACCGA CTTTAAGAAG GAAATTCTTC ATGACCGCCT TACTGGCGGA GGGGAGGGTT	TTGACCCGCC AACCCCATCA TTGGGGTAGT CTATACCCTA GATATGGGAT GTGGCCATTA CACCGGTAAT GCTTACCCCC CGAATGGGGG	GAAAGGCGCC CTGGGCTCGG GACCCGAGCC CCTAGATGGA GGATCTACCT CCTTTGACTC GGAAACTGAG AACGAGTTTG TTGCTCAAAC CCAGTGTAAC	GACCCGGAAG GCTACGACCC CGATGCTGGG ACCTTTTACC TGGAAAATGG TTCTGTCAGC AAGACAGTCG AAATTAAGCG TTTAATTCGC ATGACCAAAG	TGCGCGGAAT TTATTACACC AATAATGTGG TCAACCACAC AGTTGGTGTG TGGCCTGGCA ACCGGACCGT CTCAGTTGAC GAGTCAACTG ACTGGTTCCT
19551 19601 19651 19701	GGGGAGGCG AGACTAAGGA TCTGATTCCT TACTCTGGCT ATGAGACCGA CTTTAAGAAG GAAATTCTTC ATGACCGCCT TACTGGCGGA GGGGAGGGTT CCCCTCCCAA	TTGACCCGCC AACCCCATCA TTGGGGTAGT CTATACCCTA GATATGGGAT GTGGCCATTA CACCGGTAAT GCTTACCCCC CGAATGGGGG ACAACGTTGC TGTTGCAACG	GAAAGGCGCC CTGGGCTCGG GACCCGAGCC CCTAGATGGA GGATCTACCT CCTTTGACTC GGAAACTGAG AACGAGTTTG TTGCTCAAAC CCAGTGTAAC GGTCACATTG	GACCCGGAAG GCTACGACCC CGATGCTGGG ACCTTTTACC TGGAAAATGG TTCTGTCAGC AAGACAGTCG AAATTAAGCG TTTAATTCGC ATGACCAAAG TACTGGTTTC	TGCGCGGAAT TTATTACACC AATAATGTGG TCAACCACAC AGTTGGTGTG TGGCCTGGCA ACCGGACCGT CTCAGTTGAC GAGTCAACTG ACTGGTTCCT TGACCAAGGA
19551 19601 19651 19701	GGGGAGGCG AGACTAAGGA TCTGATTCCT TACTCTGGCT ATGAGACCGA CTTTAAGAAG GAAATTCTTC ATGACCGCCT TACTGGCGGA GGGGAGGGTT CCCCTCCCAA	TTGACCCGCC AACCCCATCA TTGGGGTAGT CTATACCCTA GATATGGGAT GTGGCCATTA CACCGGTAAT GCTTACCCC CGAATGGGGG ACAACGTTGC	GAAAGGCGCC CTGGGCTCGG GACCCGAGCC CCTAGATGGA GGATCTACCT CCTTTGACTC GGAAACTGAG AACGAGTTTG TTGCTCAAAC CCAGTGTAAC GGTCACATTG	GACCCGGAAG GCTACGACCC CGATGCTGGG ACCTTTTACC TGGAAAATGG TTCTGTCAGC AAGACAGTCG AAATTAAGCG TTTAATTCGC ATGACCAAAG TACTGGTTTC CTACCAGGGC	TGCGCGGAAT TTATTACACC AATAATGTGG TCAACCACAC AGTTGGTGTG TGGCCTGGCA ACCGGACCGT CTCAGTTGAC GAGTCAACTG ACTGGTTCCT TGACCAAGGA TTCTATATCC

Figure 27 4

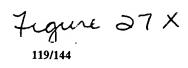
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20051	TATCCGTTCT	GGCGTCAACT	GTCGTAATGG	CAGAAAAAGT GTCTTTTTCA	AAGAAACGCT
20101	AGCGTGGGAA	ACCGCGTAGG	GTAAGAGGTC	TAACTTTATG ATTGAAATAC	AGGTACCCGC
20151	GTGAGTGTCT	GGACCCGGTT	TTGGAAGAGA	ACGCCAACTC TGCGGTTGAG	GCGGGTGCGC
20201		GAAAACTCCA	CCTAGGGTAC	CTGCTCGGGT	GGGAAGAAAT
20251	ACAAAACAAA	CTTCAGAAAC	TGCACCAGGC	TGTGCACCAG ACACGTGGTC	GGCGTGGCGC
20301	CGCAGTAGCT	TTGGCACATG	GACGCGTGCG	CCTTCTCGGC GGAAGAGCCG	GCCGTTGCGG
20351	TGTTGTATTT	CTTCGTTCGT	TGTAGTTGTT	CAGCTGCCGC	GTACCCGAGG
20401	TCACTCGTCC	TTGACTTTCG	GTAACAGTTT	GATCTTGGTT	CACCCGGTAT
20451	AAAAAACCCG	TGGATACTGT	TCGCGAAAGG	AGGCTTTGTT TCCGAAACAA	AGAGGTGTGT
20501	TCGAGCGGAC	GCGGTATCAG	TTATGCCGGC	CAGCGCTCTG	
	GTGACCTACC	GGAAACGGAC	CTTGGGCGTG	AGTTTTTGTA	GCTACCTCTT CGATGGAGAA
		CCGAAAAGAC	TGGTCGCTGA	GTTCGTCCAA	ATGGTCAAAC
		TGAGGACGCG	GCATCGCGGT	, AACGAAGAAG	GGGGCTGGCG
	ACATATTGCG	ACCTTTTCAG	GTGGGTTTCG	CATGTCCCCG	CCAACTCGGC GGTTGAGCCG
20751	CGCCTGTGGA GCGGACACCT	CTATTCTGCT GATAAGACGA	GCATGTTTCT CGTACAAAGA	CCACGCCTTT	GCCAACTGGC CGGTTGACCG

Figure 27 V.

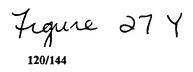
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21601	TTTGCGCCTT AAACGCGGAA	CAGAGAAGAA GTCTCTTCTT	CATGCCGCAA	GACTTGCCGG CTGAACGGCC	AAAACTGATT TTTTGACTAA
21651	GGCCGGACAG CCGGCCTGTC	GCCGCGTCGT CGGCGCAGCA	GCACGCAGCA CGTGCGTCGT	CCTTGCGTCG	GTGTTGGAGA CACAACCTCT
21701	TCTGCACCAC AGACGTGGTG	ATTTCGGCCC TAAAGCCGGG	CACCGGTTCT GTGGCCAAGA	TCACGATCTI AGTGCTAGAA	GGCCTTGCTA CCGGAACGAT

7. gure 27 W

21801		TCCTTATTTA AGGAATAAAT		
21851		CTCAGCGCAG GAGTCGCGTC	 	
21901		TGTAGGTCAC ACATCCAGTG	 	
21951		ATCATCGTCA TAGTAGCAGT	 	•
22001		GTGCTCCTCG CACGAGGAGC	 	
22051		GGTCAGGCAG CCAGTCCGTC	 	
22101		TTGTCCATCA AACAGGTAGT	 	
22151		GATCGGCACA CTAGCCGTGT		
22201		TGGGCTCTTC ACCCGAGAAG	 	
22251		TCTTCATTCA AGAAGTAAGT	 	
22301		TAGCACCGGT ATCGTGGCCA		
22351		TTTCTTCCTC AAAGAAGGAG		
22401		TTGGGAGAAG AACCCTCTTC		
22451		CGCCGAGGTC GCGGCTCCAG	 	
22501	AGCGCGTCTT TCGCGCAGAA	GTGATGAGTC CACTACTCAG	 	
22551	CATCCGCTTT GTAGGCGAAA	TTTGGGGGCG AAACCCCCGC		
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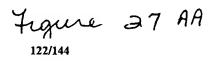
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23051				TCGCCATAGC AGCGGTATCG	
23101			=	CGCGTACCCC GCGCATGGGG	
23151		- · · · · · · · · · · · · · · · · · · ·		CCTCAACTTC GGAGTTGAAG	
23201				ACATCTTTTT TGTAGAAAAA	
23251				AGCCGAGCGG TCGGCTCGCC	
23301				TATCGCCTCG ATAGCGGAGC	
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23401				AGTCACTCTG TCAGTGAGAC	
23451				CGTACTAAAA GCATGATTTT	CGCAGCATCG GCGTCGTAGC
23501	AGGTCACCCA TCCAGTGGGT			ACCTACCCCC TGGATGGGGG	
23551				CGTGCGCAGC GCACGCGTCG	CCCTGGAGAG GGGACCTCTC
23601	GGATGCAAAT CCTACGTTTA			GGGCCTACCC CCCGGATGGG	



23701	GAGCGACGCA	AACTAATGAT	GGCCGCAGTG	CTCGTTACCG	TGGAGCTTGA
	CTCGCTGCGT	TTGATTACTA	CCGGCGTCAC	GAGCAATGGC	ACCTCGAACT
23751	GTGCATGCAG	CGGTTCTTTG	CTGACCCGGA	GATGCAGCGC	AAGCTAGAGG
	CACGTACGTC	GCCAAGAAAC	GACTGGGCCT	CTACGTCGCG	TTCGATCTCC
23801	AAACATTGCA	CTACACCTTT	CGACAGGGCT	ACGTACGCCA	GGCCTGCAAG
	TTTGTAACGT	GATGTGGAAA	GCTGTCCCGA	TGCATGCGGT	CCGGACGTTC
23851	ATCTCCAACG	TGGAGCTCTG	CAACCTGGTC	TCCTACCTTG	GAATTTTGCA
	TAGAGGTTGC	ACCTCGAGAC	GTTGGACCAG	AGGATGGAAC	CTTAAAACGT
23901	CGAAAACCGC	CTTGGGCAAA	ACGTGCTTCA	TTCCACGCTC	AAGGGCGAGG
	GCTTTTGGCG	GAACCCGTTT	TGCACGAAGT	AAGGTGCGAG	TTCCCGCTCC
23951	COCOCOCOA	CTACGTCCGC	GACTGCGTTT	ACTTATTTCT	ATGCTACACC
	TODODODODO	GATGCAGGCG	CTGACGCAAA	TGAATAAAGA	TACGATGTGG
24001	TGGCAGACGG	CCATGGGCGT	TTGGCAGCAG	TGCTTGGAGG	AGTGCAACCT
	ACCGTCTGCC	GGTACCCGCA	AACCGTCGTC	ACGAACCTCC	TCACGTTGGA
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	GGAAGTTGCT	CGCGAGGCAC	CGGCGCGTGG	ACCGCCTGTA	GTAAAAGGGG
24151		TTAAAACCCT AATTTTGGGA			TCACCAGTCA AGTGGTCAGT
24201					TCAGGAATCT AGTCCTTAGA
24251					CATTAAGTAC GTAATTCATG
24301	CGCGAATGCC	CTCCGCCGCT	TTGGGGCCAC	TGCTACCTTC	TGCAGCTAGC
	GCGCTTACGG	GAGGCGGCGA	AACCCCGGTG	ACGATGGAAG	ACGTCGATCG
24351	CAACTACCTT	GCCTACCACT	CTGACATAAT	GGAAGACGTG	AGCGGTGACG
	GTTGATGGAA	CGGATGGTGA	GACTGTATTA	CCTTCTGCAC	TCGCCACTGC
24401	GTCTACTGGA CAGATGACCT	GTGTCACTGT CACAGTGACA	CGCTGCAACC GCGACGTTGG	TATGCACCCC	GCACCGCTCC
24451	CTGGTTTGCA	ATTCGCAGCT	GCTTAACGAA	AGTCAAATTA	TCGGTACCTT
	GACCAAACGT	TAAGCGTCGA	CGAATTGCTT	TCAGTTTAAT	AGCCATGGAA
24501	TGAGCTGCAG	GGTCCCTCGC	CTGACGAAAA	GTCCGCGGCT	CCGGGGTTGA
	ACTCGACGTC	CCAGGGAGCG	GACTGCTTTT	CAGGCGCCGA	GGCCCCAACT
24551	AACTCACTCC TTGAGTGAGG	GGGGCTGTGG	ACGTCGGCTT TGCAGCCGAA	ACCTTCGCAA TGGAAGCGTT	ATTTGTACCT TAAACATGGA

Figure 27Z

24601		ACGCCCACGA			
	·CTCCTGATGG	TGCGGGTGCT	CTAATCCAAG	ATGCTTCTGG	TTAGGGCGGG
24651	GCCTAATGCG	GAGCTTACCG	CCTGCGTCAT	TACCCAGGGC	CACATTCTTG
•	CGGATTACGC	CTCGAATGGC	GGACGCAGTA	ATGGGTCCCG	GTGTAAGAAC
24701	GCCAATTGCA	AGCCATCAAC	AAAGCCCGCC	AAGAGTTTCT	GCTACGAAAG
	CGGTTAACGT	TCGGTAGTTG	TTTCGGGCGG	TTCTCAAAGA	CGATGCTTTC
24751	GGACGGGGG	TTTACTTGGA	CCCCCAGTCC	GGCGAGGAGC	TCAACCCAAT
		AAATGAACCT			
24801	CCCCCCGCCG	CCGCAGCCCT	ATCAGCAGCA	GCCGCGGGCC	CTTGCTTCCC
	GGGGGGGGG	GGCGTCGGGA	TAGTCGTCGT	CGGCGCCCGG	GAACGAAGGG
24851	ACCATCCCAC	CCAAAAAGAA	CCTCCACCTC	CCCCCCCXC	CCACCCACCA
14031		GGTTTTTCTT			
24901		TGGGACAGTC			
	CCTCCTTATG	ACCCTGTCAG	TECGTETECT	CCAAAACCTG	СТССТССТСС
24951	AGGACATGAT	GGAAGACTGG	GAGAGCCTAG	ACGAGGAAGC	TTCCGAGGTC
	TCCTGTACTA	CCTTCTGACC	CTCTCGGATC	TGCTCCTTCG	AAGGCTCCAG
25001	GAAGAGGTGT	CAGACGAAAC	ACCGTCACCC	TCGGTCGCAT	TCCCCTCGCC
		GTCTGCTTTG			
-		•		•	
25051		AAATCGGCAA			
	CCGCGGGGTC	TTTAGCCGTT	GGCCAAGGTC	GTACCGATGT	TGGAGGCGAG
25101	CTCAGGCGCC	GCCGGCACTG	CCCGTTCGCC	GACCCAACCG	TAGATGGGAC
	GAGTCCGCGG	CGGCCGTGAC	GGGCAAGCGG	CTGGGTTGGC	ATCTACCCTG
25151	ACCACTGGAA	CCAGGGCCGG	TAAGTCCAAG	CAGCCGCCGC	CGTTAGCCCA
		GGTCCCGGCC			
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23201		GTCGCGGTTC			
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25251		TTGCTTGCAA			
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	GCGAAAGAAG	AGATGGTAGT	GCCGCACCGG	AAGGGGGCAT	TGTAGGACGT
25351	TTACTACCGT	CATCTCTACA	GCCCATACTG	CACCGGCGGC	AGCGGCAGCA
		GTAGAGATGT			
25401		CCACACAGAA GGTGTGTCTT			
	101001000	GOIGIGICIT	COLLICECT	GGCCTATCGT	TCTGAGACTG
25451		AAATCCACAG			
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25501	GTCTGGCGCC	CAACGAACCC	GTATCGACCC	GCGAGCTTAG	AAACAGGATT
	-				TTTGTCCTAA



25551	TTTCCCACTC AAAGGGTGAG	TGTATGCTAT ACATACGATA	ATTTCAACAG TAAAGTTGTC	AGCAGGGGCC TCGTCCCCGG	AAGAACAAGA TTCTTGTTCT
25601		AAAAACAGGT TTTTTGTCCA			
25651		CGAAGATCAG GCTTCTAGTC			
25701		AATACTGCGC TTATGACGCG			
25751	AAGAGTTTAA	TAAGCGCGAA ATTCGCGCTT	TTGATGCAGT	AGAGGTCGCC	GGTGTGGGCC
25801		ACAACAGTCG	CGGTAATACT	CGTTCCTTTA	AGGGTGCGGG
25851	ATGTACACCT	GTTACCAGCC CAATGGTCGG	TGTTTACCCT	GAACGCCGAC	CTCGACGGGT
25901	TCTGATGAGT	ACCCGAATAA TGGGCTTATT	TGATGTACTC	GCGCCCTGGG	GTGTACTATA
25951	GGGCCCAGTT	CGGAATACGC GCCTTATGCG	CGGGTGGCTT	TGGCTTAAGA	GGACCTTGTC
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26051	GCGACGGGAC	GTGTACCAGG CACATGGTCC	TTTCAGGGCG	ÄGGGTGGTGA	CACCATGAAG
26101	GGTCTCTGCG	CCAGGCCGAA GGTCCGGCTT	CAAGTCTACT	GATTGAGTCC	CCGCGTCGAA
26151	CGCCCGCCGA	TTCGTCACAG AAGCAGTGTC	CCACGCCAGC	GGGCCCGTCC	CATATTGAGT
26201	GGACTGTTAG	TCTCCCGCTC	CATAAGTCGA	GTTGCTGCTC	
		AGAGGCAGGC	CTGCCCTGTA	AAGTCTAGCC	GCCGCGGCCG
		AGTGCGGAGC	AGTCCGTTAG	GATTGAGACG	TCTGGAGCAG
		GCGAGACCTC	CGTAACCTTG	AGACGTTAAA	TAACTCCTCA
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26451	CCGGATCAAT GGCCTAGTTA				CGGCGGACGG

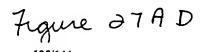
Figure 27 AB

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26551	TGGTCCACTG	TCGCCGCCAC	AAGTGCTTTG	CCCGCGACTC	CGGTGAGTTT
		AGCGGCGGTG			
	ACCAGG I GAC	AGCGGCGG1G	TICACGAAAC	00000010110	GCCAC I CALL
26601		AATTGCCCGA			
	ACGATGAAAC	TTAACGGGCT	CCTAGTATAG	CTCCCGGGCC	GCGTGCCGCA
26651	CCCCCTTACC	GCCCAGGGAG	» ccmmccccc	ጥአርርርጥር አጥጥ	CCCCACTTA
20031	••••				
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26701	CCCAGCGCCC	CCTGCTAGTT	GAGCGGGACA	GGGGACCCTG	TGTTCTCACT
	CCCTCCCCC	GGACGATCAA	CTCCCCCTCT	CCCCTGGGAC	ACAAGAGTGA
	000100000	Concomit City.	4100000101		1101,5101101-0-1
26751		ACTGTCCTAA			
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		•			
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20001		CTCATATTAT			
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	ATGAGGTAGT TGCGTCACCG	CTTTTTTGTG	GTGGGAGGAA CACACCTACC	TGGACGGCCC GCCTGACCGT	TTGCATGCTC ÄAACCAGACT
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27051	ATGAGGTAGT TGCGTCACCG ACGCAGTGGC TTTTCCGGAC	CTTTTTTGTG GCCGCTGCAC CGGCGACGTG AGACCTCAAT	GTGGGAGGAA CACACCTACC GTGTGGATGG AACTCTGTTT	TGGACGGCCC GCCTGACCGT CGGACTGGCA ACCAGAACAG	TTGCATGCTC AAACCAGACT TTTGGTCTGA GAGGTGAGCT
27051	ATGAGGTAGT TGCGTCACCG ACGCAGTGGC TTTTCCGGAC	CTTTTTTGTG GCCGCTGCAC CGGCGACGTG	GTGGGAGGAA CACACCTACC GTGTGGATGG AACTCTGTTT	TGGACGGCCC GCCTGACCGT CGGACTGGCA ACCAGAACAG	TTGCATGCTC AAACCAGACT TTTGGTCTGA GAGGTGAGCT
27051 27101	ATGAGGTAGT TGCGTCACCG ACGCAGTGGC TTTTCCGGAC AAAAGGCCTG	CTTTTTGTG GCCGCTGCAC CGGCGACGTG AGACCTCAAT TCTGGAGTTA	GTGGGAGGAA CACACCTACC GTGTGGATGG AACTCTGTTT TTGAGACAAA	TGGACGCCC GCCTGACCGT CGGACTGGCA ACCAGAACAG TGGTCTTGTC	TTGCATGCTC AAACCAGACT TTTGGTCTGA GAGGTGAGCT CTCCACTCGA
27051	ATGAGGTAGT TGCGTCACCG ACGCAGTGGC TTTTCCGGAC AAAAGGCCTG TAGAAAACCC	CTTTTTGTG GCCGCTGCAC CGGCGACGTG AGACCTCAAT TCTGGAGTTA TTAGGGTATT	GTGGGAGGAA CACACCTACC GTGTGGATGG AACTCTGTTT TTGAGACAAA AGGCCAAAGG	TGGACGCCC GCCTGACCGT CGGACTGGCA ACCAGAACAG TGGTCTTGTC CGCAGCTACT	TTGCATGCTC AAACCAGACT TTTGGTCTGA GAGGTGAGCT CTCCACTCGA GTGGGGTTTA
27051 27101	ATGAGGTAGT TGCGTCACCG ACGCAGTGGC TTTTCCGGAC AAAAGGCCTG TAGAAAACCC	CTTTTTGTG GCCGCTGCAC CGGCGACGTG AGACCTCAAT TCTGGAGTTA	GTGGGAGGAA CACACCTACC GTGTGGATGG AACTCTGTTT TTGAGACAAA AGGCCAAAGG	TGGACGCCC GCCTGACCGT CGGACTGGCA ACCAGAACAG TGGTCTTGTC CGCAGCTACT	TTGCATGCTC AAACCAGACT TTTGGTCTGA GAGGTGAGCT CTCCACTCGA GTGGGGTTTA
27051 27101	ATGAGGTAGT TGCGTCACCG ACGCAGTGGC TTTTCCGGAC AAAAGGCCTG TAGAAAACCC	CTTTTTGTG GCCGCTGCAC CGGCGACGTG AGACCTCAAT TCTGGAGTTA TTAGGGTATT	GTGGGAGGAA CACACCTACC GTGTGGATGG AACTCTGTTT TTGAGACAAA AGGCCAAAGG	TGGACGCCC GCCTGACCGT CGGACTGGCA ACCAGAACAG TGGTCTTGTC CGCAGCTACT	TTGCATGCTC AAACCAGACT TTTGGTCTGA GAGGTGAGCT CTCCACTCGA GTGGGGTTTA
27051 27101 27151	ATGAGGTAGT TGCGTCACCG ACGCAGTGGC TTTTCCGGAC AAAAGGCCTG TAGAAAACCC ATCTTTTGGG	CTTTTTGTG GCCGCTGCAC CGGCGACGTG AGACCTCAAT TCTGGAGTTA TTAGGGTATT AATCCCATAA	GTGGGAGGAA CACACCTACC GTGTGGATGG AACTCTGTTT TTGAGACAAA AGGCCAAAGG TCCGGTTTCC	TGGACGCCC GCCTGACCGT CGGACTGGCA ACCAGAACAG TGGTCTTGTC CGCAGCTACT GCGTCGATGA	TTGCATGCTC AAACCAGACT TTTGGTCTGA GAGGTGAGCT CTCCACTCGA GTGGGGTTTA CACCCCAAAT
27051 27101 27151	ATGAGGTAGT TGCGTCACCG ACGCAGTGGC TTTTTCCGGAC AAAAGGCCTG TAGAAAACCC ATCTTTTGGG TGAACAATTC	CTTTTTGTG GCCGCTGCAC CGGCGACGTG AGACCTCAAT TCTGGAGTTA TTAGGGTATT AATCCCATAA AAGCAACTCT	CACACCTACC GTGTGGATGG AACTCTGTTT TTGAGACAAA AGGCCAAAGG TCCGGTTTCC ACGGGCTATT	TGGACGGCCC GCCTGACCGT CGGACTGGCA ACCAGAACAG TGGTCTTGTC CGCAGCTACT GCGTCGATGA CTAATTCAGG	TTGCATGCTC AAACCAGACT TTTGGTCTGA GAGGTGAGCT CTCCACTCGA GTGGGGTTTA CACCCCAAAT TTTCTCTAGA
27051 27101 27151	ATGAGGTAGT TGCGTCACCG ACGCAGTGGC TTTTTCCGGAC AAAAGGCCTG TAGAAAACCC ATCTTTTGGG TGAACAATTC	CTTTTTGTG GCCGCTGCAC CGGCGACGTG AGACCTCAAT TCTGGAGTTA TTAGGGTATT AATCCCATAA AAGCAACTCT	CACACCTACC GTGTGGATGG AACTCTGTTT TTGAGACAAA AGGCCAAAGG TCCGGTTTCC ACGGGCTATT	TGGACGGCCC GCCTGACCGT CGGACTGGCA ACCAGAACAG TGGTCTTGTC CGCAGCTACT GCGTCGATGA CTAATTCAGG	TTGCATGCTC AAACCAGACT TTTGGTCTGA GAGGTGAGCT CTCCACTCGA GTGGGGTTTA CACCCCAAAT
27051 27101 27151 27201	ATGAGGTAGT TGCGTCACCG ACGCAGTGGC TTTTCCGGAC AAAAGGCCTG TAGAAAACCC ATCTTTTGGG TGAACAATTC ACTTGTTAAG	CTTTTTGTG GCCGCTGCAC CGGCGACGTG AGACCTCAAT TCTGGAGTTA TTAGGGTATT AATCCCATAA AAGCAACTCT TTCGTTGAGA	CACACCTACC GTGTGGATGG AACTCTGTTT TTGAGACAAA AGGCCAAAGG TCCGGTTTCC ACGGGCTATT TGCCCGATAA	TGGACGCCC GCCTGACCGT CGGACTGGCA ACCAGAACAG TGGTCTTGTC CGCAGCTACT GCGTCGATGA CTAATTCAGG GATTAAGTCC	TTGCATGCTC AAACCAGACT TTTGGTCTGA GAGGTGAGCT CTCCACTCGA GTGGGGTTTA CACCCCAAAT TTTCTCTAGA AAAGAGATCT
27051 27101 27151 27201	ATGAGGTAGT TGCGTCACCG ACGCAGTGGC TTTTCCGGAC AAAAGGCCTG TAGAAAACCC ATCTTTTGGG TGAACAATTC ACTTGTTAAG	CTTTTTGTG GCCGCTGCAC CGGCGACGTG AGACCTCAAT TCTGGAGTTA TTAGGGTATT AATCCCATAA AAGCAACTCT TTCGTTGAGA	CACACCTACC GTGTGGATGG AACTCTGTTT TTGAGACAAA AGGCCAAAGG TCCGGTTTCC ACGGGCTATT TGCCCGATAA	TGGACGCCC GCCTGACCGT CGGACTGGCA ACCAGAACAG TGGTCTTGTC CGCAGCTACT GCGTCGATGA CTAATTCAGG GATTAAGTCC	TTGCATGCTC AAACCAGACT TTTGGTCTGA GAGGTGAGCT CTCCACTCGA GTGGGGTTTA CACCCCAAAT TTTCTCTAGA AAAGAGATCT
27051 27101 27151 27201	ATGAGGTAGT TGCGTCACCG ACGCAGTGGC TTTTCCGGAC AAAAGGCCTG TAGAAAACCC ATCTTTTGGG TGAACAATTC ACTTGTTAAG ATCGGGGTTG	CTTTTTGTG GCCGCTGCAC CGGCGACGTG AGACCTCAAT TCTGGAGTTA TTAGGGTATT AATCCCATAA AAGCAACTCT TTCGTTGAGA GGGTTATTCT	GTGGGAGGAA CACACCTACC GTGTGGATGG AACTCTGTTT TTGAGACAAA AGGCCAAAGG TCCGGTTTCC ACGGGCTATT TGCCCGATAA CTGTCTTGTG	TGGACGCCC GCCTGACCGT CGGACTGGCA ACCAGAACAG TGGTCTTGTC CGCAGCTACT GCGTCGATGA CTAATTCAGG GATTAAGTCC ATTCTCTTTA	TTGCATGCTC AAACCAGACT TTTGGTCTGA GAGGTGAGCT CTCCACTCGA GTGGGGTTTA CACCCCAAAT TTTCTCTAGA AAAGAGATCT
27051 27101 27151 27201	ATGAGGTAGT TGCGTCACCG ACGCAGTGGC TTTTCCGGAC AAAAGGCCTG TAGAAAACCC ATCTTTTGGG TGAACAATTC ACTTGTTAAG ATCGGGGTTG	CTTTTTGTG GCCGCTGCAC CGGCGACGTG AGACCTCAAT TCTGGAGTTA TTAGGGTATT AATCCCATAA AAGCAACTCT TTCGTTGAGA GGGTTATTCT	GTGGGAGGAA CACACCTACC GTGTGGATGG AACTCTGTTT TTGAGACAAA AGGCCAAAGG TCCGGTTTCC ACGGGCTATT TGCCCGATAA CTGTCTTGTG	TGGACGCCC GCCTGACCGT CGGACTGGCA ACCAGAACAG TGGTCTTGTC CGCAGCTACT GCGTCGATGA CTAATTCAGG GATTAAGTCC ATTCTCTTTA	TTGCATGCTC AAACCAGACT TTTGGTCTGA GAGGTGAGCT CTCCACTCGA GTGGGGTTTA CACCCCAAAT TTTCTCTAGA AAAGAGATCT
27051 27101 27151 27201 27251	ATGAGGTAGT TGCGTCACCG ACGCAGTGGC TTTTCCGGAC AAAAGGCCTG TAGAAAACCC ATCTTTTGGG TGAACAATTC ACTTGTTAAG ATCGGGGTTG TAGCCCCAAC	CTTTTTGTG GCCGCTGCAC CGGCGACGTG AGACCTCAAT TCTGGAGTTA TTAGGGTATT AATCCCATAA AAGCAACTCT TTCGTTGAGA GGGTTATTCT CCCAATAAGA	GTGGGAGGAA CACACCTACC GTGTGGATGG AACTCTGTTT TTGAGACAAA AGGCCAAAGG TCCGGTTTCC ACGGGCTATT TGCCCGATAA CTGTCTTGTG GACAGAACAC	TGGACGCCC GCCTGACCGT CGGACTGGCA ACCAGAACAG TGGTCTTGTC CGCAGCTACT GCGTCGATGA CTAATTCAGG GATTAAGTCC ATTCTCTTTA TAAGAGAAAT	TTGCATGCTC AAACCAGACT TTTGGTCTGA GAGGTGAGCT CTCCACTCGA GTGGGGTTTA CACCCCAAAT TTTCTCTAGA AAAGAGATCT TTCTTATACT AAGAATATGA
27051 27101 27151 27201 27251	ATGAGGTAGT TGCGTCACCG ACGCAGTGGC TTTTCCGGAC AAAAGGCCTG TAGAAAACCC ATCTTTTGGG TGAACAATTC ACTTGTTAAG ATCGGGGTTG TAGCCCCCAAC	CTTTTTGTG GCCGCTGCAC CGGCGACGTG AGACCTCAAT TCTGGAGTTA TTAGGGTATT AATCCCATAA AAGCAACTCT TTCGTTGAGA GGGTTATTCT CCCAATAAGA TGCCTAAGGC	GTGGGAGGAA CACACCTACC GTGTGGATGG AACTCTGTTT TTGAGACAAA AGGCCAAAGG TCCGGTTTCC ACGGGCTATT TGCCCGATAA CTGTCTTGTG GACAGAACAC	TGGACGCCC GCCTGACCGT CGGACTGGCA ACCAGAACAG TGGTCTTGTC CGCAGCTACT GCGTCGATGA CTAATTCAGG GATTAAGTCC ATTCTCTTTA TAAGAGAAAT CTGTGTGCAC	TTGCATGCTC AAACCAGACT TTTGGTCTGA GAGGTGAGCT CTCCACTCGA GTGGGGTTTA CACCCCAAAT TTTCTCTAGA AAAGAGATCT TTCTTATACT AAGAATATGA ATTTGCATTT
27051 27101 27151 27201 27251	ATGAGGTAGT TGCGTCACCG ACGCAGTGGC TTTTCCGGAC AAAAGGCCTG TAGAAAACCC ATCTTTTGGG TGAACAATTC ACTTGTTAAG ATCGGGGTTG TAGCCCCCAAC	CTTTTTGTG GCCGCTGCAC CGGCGACGTG AGACCTCAAT TCTGGAGTTA TTAGGGTATT AATCCCATAA AAGCAACTCT TTCGTTGAGA GGGTTATTCT CCCAATAAGA TGCCTAAGGC	GTGGGAGGAA CACACCTACC GTGTGGATGG AACTCTGTTT TTGAGACAAA AGGCCAAAGG TCCGGTTTCC ACGGGCTATT TGCCCGATAA CTGTCTTGTG GACAGAACAC	TGGACGCCC GCCTGACCGT CGGACTGGCA ACCAGAACAG TGGTCTTGTC CGCAGCTACT GCGTCGATGA CTAATTCAGG GATTAAGTCC ATTCTCTTTA TAAGAGAAAT CTGTGTGCAC	TTGCATGCTC AAACCAGACT TTTGGTCTGA GAGGTGAGCT CTCCACTCGA GTGGGGTTTA CACCCCAAAT TTTCTCTAGA AAAGAGATCT TTCTTATACT AAGAATATGA
27051 27101 27151 27201 27251	ATGAGGTAGT TGCGTCACCG ACGCAGTGGC TTTTCCGGAC AAAAGGCCTG TAGAAAACCC ATCTTTTGGG TGAACAATTC ACTTGTTAAG ATCGGGGTTG TAGCCCCCAAC	CTTTTTGTG GCCGCTGCAC CGGCGACGTG AGACCTCAAT TCTGGAGTTA TTAGGGTATT AATCCCATAA AAGCAACTCT TTCGTTGAGA GGGTTATTCT CCCAATAAGA TGCCTAAGGC	GTGGGAGGAA CACACCTACC GTGTGGATGG AACTCTGTTT TTGAGACAAA AGGCCAAAGG TCCGGTTTCC ACGGGCTATT TGCCCGATAA CTGTCTTGTG GACAGAACAC	TGGACGCCC GCCTGACCGT CGGACTGGCA ACCAGAACAG TGGTCTTGTC CGCAGCTACT GCGTCGATGA CTAATTCAGG GATTAAGTCC ATTCTCTTTA TAAGAGAAAT CTGTGTGCAC	TTGCATGCTC AAACCAGACT TTTGGTCTGA GAGGTGAGCT CTCCACTCGA GTGGGGTTTA CACCCCAAAT TTTCTCTAGA AAAGAGATCT TTCTTATACT AAGAATATGA ATTTGCATTT
27051 27101 27151 27201 27251 27301	ATGAGGTAGT TGCGTCACCG ACGCAGTGGC TTTTCCGGAC AAAAGGCCTG TAGAAAACCC ATCTTTTGGG TGAACAATTC ACTTGTTAAG ATCGGGGTTG TAGCCCCAAC AACGCTTCTC TTGCGAAGAG	CTTTTTGTG GCCGCTGCAC CGGCGACGTG AGACCTCAAT TCTGGAGTTA TTAGGGTATT AATCCCATAA AAGCAACTCT TTCGTTGAGA GGGTTATTCT CCCAATAAGA TGCCTAAGGC ACGGATTCCG	GTGGGAGGAA CACACCTACC GTGTGGATGG AACTCTGTTT TTGAGACAAA AGGCCAAAGG TCCGGTTTCC ACGGGCTATT TGCCCGATAA CTGTCTTGTG GACAGAACAC TCGCCGCCTG AGCGGCGGAC	TGGACGGCCC GCCTGACCGT CGGACTGGCA ACCAGAACAG TGGTCTTGTC CGCAGCTACT GCGTCGATGA CTAATTCAGG GATTAAGTCC ATTCTCTTTA TAAGAGAAAT CTGTGTGCAC GACACACGTG	TTGCATGCTC AAACCAGACT TTTGGTCTGA GAGGTGAGCT CTCCACTCGA GTGGGGTTTA CACCCCAAAT TTTCTCTAGA AAAGAGATCT TTCTTATACT AAGAATATGA ATTTGCATTT TAAACGTAAA
27051 27101 27151 27201 27251 27301	ATGAGGTAGT TGCGTCACCG ACGCAGTGGC TTTTCCGGAC AAAAGGCCTG TAGAAAACCC ATCTTTTGGG TGAACAATTC ACTTGTTAAG ATCGGGGTTG TAGCCCCCAAC AACGCTTCTC TTGCGAAGAG	CTTTTTGTG GCCGCTGCAC CGGCGACGTG AGACCTCAAT TCTGGAGTTA TTAGGGTATT AATCCCATAA AAGCAACTCT TTCGTTGAGA GGGTTATTCT CCCAATAAGA TGCCTAAGGC ACGGATTCCG	GTGGGAGGAA CACACCTACC GTGTGGATGG AACTCTGTTT TTGAGACAAA AGGCCAAAGG TCCGGTTTCC ACGGGCTATT TGCCCGATAA CTGTCTTGTG GACAGAACAC TCGCCGCCTG AGCGGCGGAC	TGGACGCCC GCTGACCGT CGGACTGCCA ACCAGAACAG TGGTCTTGTC CGCAGCTACT GCGTCGATGA CTAATTCAGG GATTAAGTCC ATTCTCTTTA TAAGAGAAAT CTGTGTGCAC GACACACGTG ACCCAAGATG	AAACCAGACT TTTGGTCTGA GAGGTGAGCT CTCCACTCGA GTGGGGTTTA CACCCCAAAT TTTCTCTAGA AAAGAGATCT TTCTTATACT AAGAATATGA ATTTGCATTT TAAACGTAAA ATTAGGTACA
27051 27101 27151 27201 27251 27301	ATGAGGTAGT TGCGTCACCG ACGCAGTGGC TTTTCCGGAC AAAAGGCCTG TAGAAAACCC ATCTTTTGGG TGAACAATTC ACTTGTTAAG ATCGGGGTTG TAGCCCCCAAC AACGCTTCTC TTGCGAAGAG	CTTTTTGTG GCCGCTGCAC CGGCGACGTG AGACCTCAAT TCTGGAGTTA TTAGGGTATT AATCCCATAA AAGCAACTCT TTCGTTGAGA GGGTTATTCT CCCAATAAGA TGCCTAAGGC ACGGATTCCG	GTGGGAGGAA CACACCTACC GTGTGGATGG AACTCTGTTT TTGAGACAAA AGGCCAAAGG TCCGGTTTCC ACGGGCTATT TGCCCGATAA CTGTCTTGTG GACAGAACAC TCGCCGCCTG AGCGGCGGAC	TGGACGCCC GCTGACCGT CGGACTGCCA ACCAGAACAG TGGTCTTGTC CGCAGCTACT GCGTCGATGA CTAATTCAGG GATTAAGTCC ATTCTCTTTA TAAGAGAAAT CTGTGTGCAC GACACACGTG ACCCAAGATG	TTGCATGCTC AAACCAGACT TTTGGTCTGA GAGGTGAGCT CTCCACTCGA GTGGGGTTTA CACCCCAAAT TTTCTCTAGA AAAGAGATCT TTCTTATACT AAGAATATGA ATTTGCATTT TAAACGTAAA
27051 27101 27151 27201 27251 27301 27351	ATGAGGTAGT TGCGTCACCG ACGCAGTGGC TTTTCCGGAC AAAAGGCCTG TAGAAAACCC ATCTTTTGGG TGAACAATTC ACTTGTTAAG ATCGGGGTTG TAGCCCCAAC AACGCTTCTC TTGCGAAGAG ATTGTCAGCT TTACAGCT	CTTTTTGTG GCCGCTGCAC CGGCGACGTG AGACCTCAAT TCTGGAGTTA TTAGGGTATT AATCCCATAA AAGCAACTCT TTCGTTGAGA GGGTTATTCT CCCAATAAGA TGCCTAAGGC ACGGATTCCG TTTTAAACGC AAAATTTGCG	CTGGGAGGAA CACACCTACC GTGTGGATGG AACTCTGTTT TTGAGACAAA AGGCCAAAGG TCCGGTTTCC ACGGGCTATT TGCCCGATAA CTGTCTTGTG GACAGAACAC TCGCCGCCTG AGCGGCGAC TGGGGTCGCC ACCCCAGCGG	TGGACGCCC GCCTGACCGT CGGACTGCCA ACCAGAACAG TGGTCTTGTC CGCAGCTACT GCGTCGATGA CTAATTCAGG GATTAAGTCC ATTCTCTTTA TAAGAGAAAT CTGTGTGCAC GACACACGTG ACCCAAGATG	AAACCAGACT TTTGGTCTGA GAGGTGAGCT CTCCACTCGA GTGGGGTTTA CACCCCAAAT TTTCTCTAGA AAAGAGATCT TTCTTATACT AAGAATATGA ATTGCATTT TAAACGTAAA ATTAGGTACA TAATCCATGT
27051 27101 27151 27201 27251 27301 27351	ATGAGGTAGT TGCGTCACCG ACGCAGTGGC TTTTCCGGAC AAAAGGCCTG TAGAAAACCC ATCTTTTGGG TGAACAATTC ACTTGTTAAG ATCGGGGTTG TAGCCCCAAC AACGCTTCTC TTGCGAAGAG ATTGTCAGCT TAACAGTCGA	CTTTTTGTG GCCGCTGCAC CGGCGACGTG AGACCTCAAT TCTGGAGTTA TTAGGGTATT AATCCCATAA AAGCAACTCT TTCGTTGAGA GGGTTATTCT CCCAATAAGA TGCCTAAGGC ACGGATTCCG TTTTAAACGC AAAATTTGCG	CTGGGAGGAA CACACCTACC GTGTGGATGG AACTCTGTTT TTGAGACAAA AGGCCAAAGG TCCGGTTTCC ACGGGCTATT TGCCCGATAA CTGTCTTGTG GACAGAACAC TCGCCGCCTG AGCGGCGAC TGGGGTCGCC ACCCCAGCGG CTTGCGTCAG	TGGACGCCC GCCTGACCGT CGGACTGCCA ACCAGAACAG TGGTCTTGTC CGCAGCTACT GCGTCGATGA CTAATTCAGG GATTAAGTCC ATTCTCTTTA TAAGAGAAAT CTGTGTGCAC GACACACGTG ACCCAAGATG TGGGTTCTAC	AAACCAGACT TTTGGTCTGA GAGGTGAGCT CTCCACTCGA GTGGGGTTTA CACCCCAAAT TTTCTCTAGA AAAGAGATCT TTCTTATACT AAGAATATGA ATTTGCATTT TAAACGTAAA ATTAGGTACA TAATCCATGT CACCCAAAAG
27051 27101 27151 27201 27251 27301 27351	ATGAGGTAGT TGCGTCACCG ACGCAGTGGC TTTTCCGGAC AAAAGGCCTG TAGAAAACCC ATCTTTTGGG TGAACAATTC ACTTGTTAAG ATCGGGGTTG TAGCCCCAAC AACGCTTCTC TTGCGAAGAG ATTGTCAGCT TAACAGTCGA	CTTTTTGTG GCCGCTGCAC CGGCGACGTG AGACCTCAAT TCTGGAGTTA TTAGGGTATT AATCCCATAA AAGCAACTCT TTCGTTGAGA GGGTTATTCT CCCAATAAGA TGCCTAAGGC ACGGATTCCG TTTTAAACGC AAAATTTGCG	CTGGGAGGAA CACACCTACC GTGTGGATGG AACTCTGTTT TTGAGACAAA AGGCCAAAGG TCCGGTTTCC ACGGGCTATT TGCCCGATAA CTGTCTTGTG GACAGAACAC TCGCCGCCTG AGCGGCGAC TGGGGTCGCC ACCCCAGCGG CTTGCGTCAG	TGGACGCCC GCCTGACCGT CGGACTGCCA ACCAGAACAG TGGTCTTGTC CGCAGCTACT GCGTCGATGA CTAATTCAGG GATTAAGTCC ATTCTCTTTA TAAGAGAAAT CTGTGTGCAC GACACACGTG ACCCAAGATG TGGGTTCTAC	AAACCAGACT TTTGGTCTGA GAGGTGAGCT CTCCACTCGA GTGGGGTTTA CACCCCAAAT TTTCTCTAGA AAAGAGATCT TTCTTATACT AAGAATATGA ATTGCATTT TAAACGTAAA ATTAGGTACA TAATCCATGT

Figure 27AC

WO 02/022080					PCT/US01/28861		
27451		AGGAGCCAGC TCCTCGGTCG					
27501	TGAGTGCACC	ACTCTTATAA TGAGAATATT					

27551 TTCGCCACAA AAACAAAATT GGCAAGTATG CTGTTTATGC TATTTGGCAG AAGCGGTGTT TTTGTTTTAA CCGTTCATAC GACAAATACG ATAAACCGTC 27601 CCAGGTGACA CTACAGAGTA TAATGTTACA GTTTTCCAGG GTAAAAGTCA GGTCCACTGT GATGTCTCAT ATTACAATGT CAAAAGGTCC CATTTTCAGT 27651 TAAAACTTTT ATGTATACTT TTCCATTTTA TGAAATGTGC GACATTACCA ATTTTGAAAA TACATATGAA AAGGTAAAAT ACTTTACACG CTGTAATGGT 27701 TGTACATGAG CAAACAGTAT AAGTTGTGGC CCCCACAAAA TTGTGTGGAA ACATGTACTC GTTTGTCATA TTCAACACCG GGGGTGTTTT AACACACCTT 27751 AACACTGGCA CTTTCTGCTG CACTGCTATG CTAATTACAG TGCTCGCTTT TTGTGACCGT GAAAGACGAC GTGACGATAC GATTAATGTC ACGAGCGAAA 27801 GGTCTGTACC CTACTCTATA TTAAATACAA AAGCAGACGC AGCTTTATTG CCAGACATGG GATGAGATAT AATTTATGTT TTCGTCTGCG TCGAAATAAC 27851 AGGAAAAGAA AATGCCTTAA TTTACTAAGT TACAAAGCTA ATGTCACCAC TCCTTTTCTT TTACGGAATT AAATGATTCA ATGTTTCGAT TACAGTGGTG 27901 TAACTGCTTT ACTCGCTGCT TGCAAAACAA ATTCAAAAAG TTAGCATTAT ATTGACGAAA TGAGCGACGA ACGTTTTGTT TAAGTTTTTC AATCGTAATA 27951 AATTAGAATA GGATTTAAAC CCCCCGGTCA TTTCCTGCTC AATACCATTC TTAATCTTAT CCTAAATTTG GGGGGCCAGT AAAGGACGAG TTATGGTAAG 28001 CCCTGAACAA TTGACTCTAT GTGGGATATG CTCCAGCGCT ACAACCTTGA GGGACTTGTT AACTGAGATA CACCCTATAC GAGGTCGCGA TGTTGGAACT 28051 AGTCAGGCTT CCTGGATGTC AGCATCTGAC TTTGGCCAGC ACCTGTCCCG TCAGTCCGAA GGACCTACAG TCGTAGACTG AAACCGGTCG TGGACAGGGC 28101 CGGATTTGTT CCAGTCCAAC TACAGCGACC CACCCTAACA GAGATGACCA GCCTAAACAA GGTCAGGTTG ATGTCGCTGG GTGGGATTGT CTCTACTGGT 28151 ACACAACCAA CGCGGCCGCC GCTACCGGAC TTACATCTAC CACAAATACA TGTGTTGGTT GCGCCGGCGG CGATGGCCTG AATGTAGATG GTGTTTATGT 28201 CCCCAAGTTT CTGCCTTTGT CAATAACTGG GATAACTTGG GCATGTGGTG GGGGTTCAAA GACGGAAACA GTTATTGACC CTATTGAACC CGTACACCAC 28251 GTTCTCCATA GCGCTTATGT TTGTATGCCT TATTATTATG TGGCTCATCT CAAGAGGTAT CGCGAATACA AACATACGGA ATAATAATAC ACCGAGTAGA 28301 GCTGCCTAAA GCGCAAACGC GCCCGACCAC CCATCTATAG TCCCATCATT CGACGGATTT CGCGTTTGCG CGGGCTGGTG GGTAGATATC AGGGTAGTAA 28351 GTGCTACACC CAAACAATGA TGGAATCCAT AGATTGGACG GACTGAAACA CACGATGTGG GTTTGTTACT ACCTTAGGTA TCTAACCTGC CTGACTTTGT



28451		CTGACCCTTG GACTGGGAAC			
28501		TCACATCGAA			
20001		AGTGTAGCTT			
28551		GATTTGTCAC CTAAACAGTG			
28601		TTTATCCAGT AAATAGGTCA			
28651	****	CCATCCCCAG GGTAGGGGTC			
28701		AATTATGAAA			
	TCTTAAGAAA	TTAATACTTT	AAATGACACT	GAAAAGACGA	CTAATAAACG
28751		CGTTTTGTTC GCAAAACAAG			
28801		ACTCGTATAT TGAGCATATA			
28851		CGAAGCCTGG			
		GCTTCGGACC			
28901		TCTTAGCCCT AGAATCGGGA			
28951		GATGCCATGA CTACGGTACT			-
29001	* *	ACAAGTTGTT TGTTCAACAA			
29051		CTCCCACCCC GAGGGTGGGG			
29101		TGACACCCTA ACTGTGGGAT			
29151					AGCGCATGAA TCGCGTACTT
29201					AGGGGTATCT TCCCCATAGA
29251	TTTGTCTCGT AAACAGAGCA				TACCACCGGA ATGGTGGCCT
29301					TGGTGGTCAT ACCACCAGTA

Figure 27 A E

29401	GCTGCATTCA CGACGTAAGT		AGGATCTCTG TCCTAGAGAC	
29451	AAGACCCTGT TTCTGGGACA		CCCTTTAACT GGGAAATTGA	
29501		 	TTAGCAAATT AATCGTTTAA	•
29551		 	CAGCTCTGGT GTCGAGACCA	
29601			AAATGGAATG TTTACCTTAC	
29651			TCATGTTGTT AGTACAACAA	
29701			CCCGTGTATC GGGCACATAG	
29751			TACTCCTCCC ATGAGGAGGG	
29801			TACTCTCTTT ATGAGAGAAA	
29851			GCGCTCAAAA CGCGAGTTTT	
29901			CTCCCAAAAT GAGGGTTTTA	
29951			ACATAAACCT TGTATTTGGA	
	GCACCCCTCA CGTGGGGAGT			
30051			GCAATCACAG CGTTAGTGTC	
30101	CCGTGCACGA GGCACGTGCT			CCTCACAGTG GGAGTGTCAC
30151	TCAGAAGGAA AGTCTTCCTT			CCACCACCGA GGTGGTGGCT
30201	TAGCAGTACC ATCGTCATGG			ACTGCCACTG TGACGGTGAC
30251				AAATGGAAAA TTTACCTTTT

Figure 27 AF

30351					ACTTCCTTGC
	AAACTGGCAT	CGTTGACCAG	GTCCACACTG	ATAATTATTA	TGAAGGAACG
30401		TACTGGAGCC ATGACCTCGG			
	TITGATTICA	ATGACCTCGG	AACCCAAAAC	TAAGTGTTCC	GTTATACGTT
30451	CTTAATGTAG	CAGGAGGACT	AAGGATTGAT	TCTCAAAACA	GACGCCTTAT
	GAATTACATC	GTCCTCCTGA	TTCCTAACTA	AGAGTTTTGT	CTGCGGAATA
30501	ACTTGATGTT	AGTTATCCGT	TTGATGCTCA	AAACCAACTA	AATCTAAGAC
	TGAACTACAA	TCAATAGGCA	AACTACGAGT	TTTGGTTGAT	TTAGATTCTG
30551		CCCTCTTTTT			
	ATCCTGTCCC	GGGAGAAAA	TATTTGAGTC	GGGTGTTGAA	CCTATAATTG
30601	TACAACAAAG	GCCTTTACTT	GTTTACAGCT	TCAAACAATT	CCAAAAAGCT
	ATGTTGTTTC	CGGAAATGAA	CAAATGTCGA	AGTTTGTTAA	GGTTTTTCGA
30651	TGAGGTTAAC	CTAAGCACTG	CCAAGGGGTT	GATGTTTGAC	GCTACAGCCA
	ACTCCAATTG	GATTCGTGAC	GGTTCCCCAA	CTACAAACTG	CGATGTCGGT
20701			•		
30701		TGCAGGAGAT			
	ATCGGTAATT	ACGTCCTCTA	CCCGAACTTA	AACCAAGTGG	ATTACGTGGT
30751	AACACAAATC	CCCTCAAAAC	AAAAATTGGC	CATGGCCTAG	AATTTGATTC
	TTGTGTTTAG	GGGAGTTTTG	TTTTTAACCG	GTACCGGATC	TTÄAACTAAG
30801	AAACAAGGCT	ATGGTTCCTA	AACTAGGAAC	TGGCCTTAGT	TTTGACAGCA
	TTTGTTCCGA	TACCAAGGAT	TTGATCCTTG	ACCGGAATCA	AAACTGTCGT
30851	CAGGTGCCAT	TACAGTAGGA	AACAAAAATA	ATGATAAGCT	AACTTTGTGG
	GTCCACGGTA	ATGTCATCCT	TTGTTTTTAT	TACTATTCGA	TTGAAACACC
30901	ACCACACCAG	CTCCATCTCC	TAACTGTAGA	СТАДАТССАС	ACAAACATCC
30302		GAGGTAGAGG			
	1001010010	a.gornonoc	mi i onemici	GATTIACGIC	iciticinco
30951		TTGGTCTTAA			
	ATTTGAGTGA	AACCAGAATT	GTTTTACACC	GTCAGTTTAT	GAACGATGTC
31001		GGCTGTTAAA			
	AAAGTCAAAA	CCGACAATTT	CCGTCAAACC	GAGGTTATAG	ACCTTGTCAA
31051	CAAAGTGCTC	ATCTTATTAT	AAGATTTGAC	GAAAATGGAG	TGCTACTAAA
		TAGAATAATA			=
31101	CAATTCCTTC	CTGGACCCAG	AATATTGGAA	CTTTAGAAAT	GGAGATCTTA
	GTTAAGGAAG	GACCTGGGTC	TTATAACCTT	GAAATCTTTA	CCTCTAGAAT
31151	CTGAAGGCAC	AGCCTATACA	AACGCTGTTG	GATTTATGCC	TAACCTATCA
	GACTTCCGTG	TCGGATATGT	TTGCGACAAC	CTAAATACGG	ATTGGATAGT
31201	GCTTATCCAA	אארריי	T2222CTCCC		ውጥርጥር አርጥር አ
21201		TTAGAGTGCC			

Figure 27 AG

WO 02/022080 PCT/US01/28861 31251 AGTTTACTTA AACGGAGACA AAACTAAACC TGTAACACTA ACCATTACAC TCAAATGAAT TTGCCTCTGT TTTGATTTGG ACATTGTGAT TGGTAATGTG 313C1 TAAACGGTAC ACAGGAAACA GGAGACACAA CTCCAAGTGC ATACTCTATG ATTTGCCATG TGTCCTTTGT CCTCTGTGTT GAGGTTCACG TATGAGATAC 31351 TCATTTTCAT GGGACTGGTC TGGCCACAAC TACATTAATG AAATATTTGC AGTAAAAGTA CCCTGACCAG ACCGGTGTTG ATGTAATTAC TTTATAAACG 31401 CACATCCTCT TACACTTTTT CATACATTGC CCAAGAATAA AGAATCGTTT GTGTAGGAGA ATGTGAAAAA GTATGTAACG GGTTCTTATT TCTTAGCAAA 31451 GTGTTATGTT TCAACGTGTT TATTTTTCAA TTGCAGAAAA TTTCAAGTCA CACAATACAA AGTTGCACAA ATAAAAAGTT AACGTCTTTT AAAGTTCAGT 31501 TTTTCATTC AGTAGTATAG CCCCACCACC ACATAGCTTA TACAGATCAC AAAAAGTAAG TCATCATATC GGGGTGGTGG TGTATCGAAT ATGTCTAGTG 31551 CGTACCTTAA TCAAACTCAC AGAACCCTAG TATTCAACCT GCCACCTCCC GCATGGAATT AGTTTGAGTG TCTTGGGATC ATAAGTTGGA CGGTGGAGGG 31601 TCCCAACACA CAGAGTACAC AGTCCTTTCT CCCCGGCTGG CCTTAAAAAG AGGGTTGTGT GTCTCATGTG TCAGGAAAGA GGGGCCGACC GGAATTTTTC 31651 CATCATATCA TGGGTAACAG ACATATTCTT AGGTGTTATA TTCCACACGG GTAGTATAGT ACCCATTGTC TGTATAAGAA TCCACAATAT AAGGTGTGCC 31701 TTTCCTGTCG AGCCAAACGC TCATCAGTGA TATTAATAAA CTCCCCGGGC AAAGGACAGC TCGGTTTGCG AGTAGTCACT ATAATTATTT GAGGGGCCCG 31751 AGCTCACTTA AGTTCATGTC GCTGTCCAGC TGCTGAGCCA CAGGCTGCTG TCGAGTGAAT TCAAGTACAG CGACAGGTCG ACGACTCGGT GTCCGACGAC 31801 TCCAACTTGC GGTTGCTTAA CGGGCGGCGA AGGAGAAGTC CACGCCTACA AGGTTGAACG CCAACGAATT GCCCGCCGCT TCCTCTTCAG GTGCGGATGT 31851 TGGGGGTAGA GTCATAATCG TGCATCAGGA TAGGGCGGTG GTGCTGCAGC ACCCCCATCT CAGTATTAGC ACGTAGTCCT ATCCCGCCAC CACGACGTCG 31901 AGCGCGCGAA TAAACTGCTG CCGCCGCCGC TCCGTCCTGC AGGAATACAA TCGCGCGCTT ATTTGACGAC GGCGGCGGCG AGGCAGGACG TCCTTATGTT 31951 CATGGCAGTG GTCTCCTCAG CGATGATTCG CACCGCCCGC AGCATAAGGC GTACCGTCAC CAGAGGAGTC GCTACTAAGC GTGGCGGGCG TCGTATTCCG 32001 GCCTTGTCCT CCGGGCACAG CAGCGCACCC TGATCTCACT TAAATCAGCA CGGAACAGGA GGCCCGTGTC GTCGCGTGGG ACTAGAGTGA ATTTAGTCGT 32051 CAGTAACTGC AGCACAGCAC CACAATATTG TTCAAAATCC CACAGTGCAA GTCATTGACG TCGTGTCGTG GTGTTATAAC AAGTTTTAGG GTGTCACGTT 32101 GGCGCTGTAT CCAAAGCTCA TGGCGGGGAC CACAGAACCC ACGTGGCCAT CCGCGACATA GGTTTCGAGT ACCGCCCCTG GTGTCTTGGG TGCACCGGTA 32151 CATACCACAA GCGCAGGTAG ATTAAGTGGC GACCCCTCAT AAACACGCTG

Figure 27AH

GTATGGTGTT CGCGTCCATC TAATTCACCG CTGGGGAGTA TTTGTGCGAC

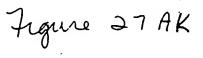
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32301		AACCTGCCCG TTGGACGGGC			
32351		AGTGGAGAGC TCACCTCTCG			
32401		TCAATGTTGG AGTTACAACC			
32451		AAGCTCCTCC TTCGAGGAGG	· · · - · -		
32501		TCAGCGTAAA AGTCGCATTT			
32551		TGCATTGTCA ACGTAACAGT			
32601		GGTAGCGCGG CCATCGCGCC			
32651		GAGTGCGCCG CTCACGCGGC		-,	
32701		GGAACGCCGG CCTTGCGGCC			
32751		GACAAACAGA CTGTTTGTCT			
32801		TAGTTGTAGT ATCAACATCA			
32851		GGGTTCTATG CCCAAGATAC			
32901		CCGCAGAATA GGCGTCTTAT			
32951					ACCATGTTTT TGGTACAAAA
33001		CCAAAAGATT GGTTTTCTAA			GATCTATTAA CTAGATAATT
33051	GTGAACGCGC CACTTGCGCG				GCCAAAGAAC CGGTTTCTTG
33101	AGATAATGGC TCTATTACCG				AAGGCAAACG TTCCGTTTGC

Figure 27 AI

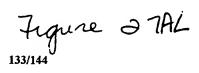
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33251	GCCACCTTCT CGGTGGAAGA			CCCGAATATT GGGCTTATAA	
33301				ACCTTCAGCC TGGAAGTCGG	
33351				CAGACCTGTA GTCTGGACAT	
33401	AAGCGGAACA TTCGCCTTGT	_		CCGTAGGTCC GGCATCCAGG	
33451				GGACCAGCGC CCTGGTCGCG	
33501				CTGATTATGA GACTAATACT	
33,551	CGGAGÇTATG GCCTCGATAC	CTAACCAGCG GATTGGTCGC	TAGCCCCGAT ATCGGGGCTA	GTAAGCTTGT CATTCGAACA	TGCATGGGCG ACGTACCCGC
33601				AATCAGGCAA TTAGTCCGTT	
33651				TGCAGATAAA ACGTCTATTT	
33701				TTTTCTCTCA AAAAGAGAGT	
33751				ACAAAAAAAC TGTTTTTTTG	
33801				CCCTTATAAG GGGAATATTC	
33851				AACTGGTCAC TTGACCAGTG	
33901	AAGCACCACC TTCGTGGTGG				ATGTAAGACT TACATTCTGA
33951	CGGTAAACAC GCCATTTGTG				AAAGCGACCG TTTCGCTGGC
34001	AAATAGCCCG TTTATCGGGC			CGTAGAGACA GCATCTCTGT	
34051	CCCCATAGGA GGGGTATCCT			AGAGAAAAAC TCTCTTTTTG	

Ligure 27AJ

34151		CTTCCACAGC GAAGGTGTCG			
34201		CTATTAAAAA GATAATTTTT			
34251		TAAAAAAGGG ATTTTTTCCC	-		
34301	AAAAATGACG TTTTTACTGC	TAACGGTTAA ATTGCCAATT			
34351		GCCCAGAAAC CGGGTCTTTG			
34401		CGTTTTCCCA GCAAAAGGGT			
34451		ACACATACAA TGTGTATGTT			
34501		ACGCCCCGCG TGCGGGGCGC			
					PacI
34551		CAATCCAAAA GTTAGGTTTT			
34601		TGCGACGCGA ACGCTGCGCT	••		=
34651			a. ====================================	mmcc	
34031		GCGGCATCGG CGCCGTAGCC			TGCTGTCCAG ACGACAGGTC
34701	GAGCGAAGGC GCAGGTAGAT		CTACGGGCGC AGGGACAGCT	AACGTCCGGT TCAAGGCCAG	ACGACAGGTC CAAAAGGCCA
	GAGCGAAGGC GCAGGTAGAT CGTCCATCTA GGAACCGTAA	CGCCGTAGCC GACGACCATC	CTACGGGCGC AGGGACAGCT TCCCTGTCGA TTGCTGGCGT	AACGTCCGGT TCAAGGCCAG AGTTCCGGTC TTTTCCATAG	ACGACAGGTC CAAAAGGCCA GTTTTCCGGT GCTCCGCCCC
34701 34751	GAGCGAAGGC GCAGGTAGAT CGTCCATCTA GGAACCGTAA CCTTGGCATT CCTGACGAGC	CGCCGTAGCC GACGACCATC CTGCTGGTAG AAAGGCCGCG TTTCCGGCGC	CTACGGGCGC AGGGACAGCT TCCCTGTCGA TTGCTGGCGT AACGACCGCA TCGACGCTCA	AACGTCCGGT TCAAGGCCAG AGTTCCGGTC TTTTCCATAG AAAAGGTATC AGTCAGAGGT	ACGACAGGTC CAAAAGGCCA GTTTTCCGGT GCTCCGCCCC CGAGGCGGGG GGCGAAACCC
34701 34751 34801	GAGCGAAGGC GCAGGTAGAT CGTCCATCTA GGAACCGTAA CCTTGGCATT CCTGACGAGC GGACTGCTCG GACAGGACTA	CGCCGTAGCC GACGACCATC CTGCTGGTAG AAAGGCCGCG TTTCCGGCGC ATCACAAAAA TAGTGTTTTT	CTACGGGCGC AGGGACAGCT TCCCTGTCGA TTGCTGGCGT AACGACCGCA TCGACGCTCA AGCTGCGAGT AGGCGTTTCC	AACGTCCGGT TCAAGGCCAG AGTTCCGGTC TTTTCCATAG AAAAGGTATC AGTCAGAGGT TCAGTCTCCA CCCTGGAAGC	ACGACAGGTC CAAAAGGCCA GTTTTCCGGT GCTCCGCCC CGAGGCGGGG GGCGAAACCC CCGCTTTGGG TCCCTCGTGC
34701 34751 34801 34851	GAGCGAAGGC GCAGGTAGAT CGTCCATCTA GGAACCGTAA CCTTGGCATT CCTGACGAGC GGACTGCTCG GACAGGACTA CTGTCCTGAT	CGCCGTAGCC GACGACCATC CTGCTGGTAG AAAGGCCGCG TTTCCGGCGC ATCACAAAAA TAGTGTTTTT TAAAGATACC ATTTCTATGG	CTACGGGCGC AGGGACAGCT TCCCTGTCGA TTGCTGGCGT AACGACCGCA TCGACGCTCA AGCTGCGAGT AGGCGTTTCC TCCGCAAAGG CCGCTTACCG	AACGTCCGGT TCAAGGCCAG AGTTCCGGTC TTTTCCATAG AAAAGGTATC AGTCAGAGGT TCAGTCTCCA CCCTGGAAGC GGGACCTTCG GATACCTGTC	ACGACAGGTC CAAAAGGCCA GTTTTCCGGT GCTCCGCCCC CGAGGCGGGG GGCGAAACCC CCGCTTTGGG TCCCTCGTGC AGGGAGCACG CGCCTTTCTC
34701 34751 34801 34851 34901	GAGCGAAGGC GCAGGTAGAT CGTCCATCTA GGAACCGTAA CCTTGGCATT CCTGACGAGC GGACTGCTCG GACAGGACTA CTGTCCTGAT CGTCTCCTGT CGAGAGGACA CCTTCGGGAA	CGCCGTAGCC GACGACCATC CTGCTGGTAG AAAGGCCGCG TTTCCGGCGC ATCACAAAAA TAGTGTTTTT TAAAGATACC ATTTCTATGG TCCGACCCTG AGGCTGGGAC	CTACGGGCGC AGGGACAGCT TCCCTGTCGA TTGCTGGCGT AACGACCGCA TCGACGCTCA AGCTGCGAGT AGGCGTTTCC TCCGCAAAGG CCGCTTACCG GGCGAATGGC TTCTCATAGC	AACGTCCGGT TCAAGGCCAG AGTTCCGGTC TTTTCCATAG AAAAGGTATC AGTCAGAGGT TCAGTCTCCA CCCTGGAAGC GGGACCTTCG GATACCTGTC CTATGGACAG TCACGCTGTA	ACGACAGGTC CAAAAGGCCA GTTTTCCGGT GCTCCGCCC CGAGGCGGGG GGCGAAACCC CCGCTTTGGG TCCCTCGTGC AGGGAGCACG CGCCTTTCTC GCGGAAAGAG



	AAGTCGGGCT	GGCGACGCGG	AATAGGCCAT	TGATAGCAGA	ACTCAGGTTG
35101		ACGACTTATC TGCTGAATAG			
35151	TAGCAGAGCG	AGGTATGTAG	GCGGTGCTAC	AGAGTTCTTG	AAGTGGTGGC
	ATCGTCTCGC	TCCATACATC	CGCCACGATG	TCTCAAGAAC	TTCACCACCG
35201		CTACACTAGA			-
	GATTGATGCC	GATGTGATCT	TCCTGTCATA	AACCATAGAC	GCGAGACGAC
35251		CCTTCGGAAA			•
		GGAAGCCTTT			
35301		GGTAGCGGTG CCATCGCCAC			
	1166166CGA	CCATCGCCAC	CAMMONANCA	AACGIICGIC	GICIAAIGCG
35351		AGGATCTCAA			
	CGTCTTTTTT	TCCTAGAGTT	CTTCTAGGAA	ACTAGAAAAG	ATGCCCCAGA
35401	GACGCTCAGT	GGAACGAAAA	CTCACGTTAA	GGGATTTTGG	TCATGAGATT
	CTGCGAGTCA	CCTTGCTTTT	GAGTGCAATT	CCCTAAAACC	AGTACTCTAA
35451		ATCTTCACCT			
	TAGTTTTTCC	TAGAAGTGGA	TCTAGGAAAA	TTTAGTTAGA	TTTCATATAT
35501		TGGTCTGACA			
	ACTCATTTGA	ACCAGACTGT	CAATGGTTAC	GAATTAGTCA	CTCCGTGGAT
35551		CTGTCTATTT			
	AGAGTCGCTA	GACAGATAAA	GCAAGTAGGT	ATCAACGGAC	TGAGGGGCAG
35601		CTACGATACG	· -		
	CACATCTATT	GATGCTATGC	CCTCCCGAAT	GGTAGACCGG	GGTCACGACG
35651		CGAGACCCAC			
	TTACTATGGC	GCTCTGGGTG	CGAGTGGCCG	AGGICTAAAT	AGICGITATI
35701	ACCAGCCAGC	CGGAAGGGCC	GAGCGCAGAA	GTGGTCCTGC	AACTTTATCC
	TGGTCGGTCG	GCCTTCCCGG	CTCGCGTCTT	CACCAGGACG	TTGAAATAGG
35751					TAAGTAGTTC
	CGGAGGTAGG	TCAGATAATT	AACAACGGCC	CTTCGATCTC	ATTCATCAAG
35801					GGCATCGTGG
	CGGTCAATTA	TCAAACGCGT	TGCAACAACG	GTAACGATGT	CCGTAGCACC
35851					TTCCCAACGA
	ACAGTGCGAG	CAGCAAACCA	TACCGAAGTA	AGTCGAGGCC	AAGGGTTGCT
35901					CGGTTAGCTC
•					GCCAATCGAG
35951					GTGTTATCAC
	GAAGCCAGGA	GGCTAGCAAC	AGTCTTCATT	CAACCGGCGT	CACAATAGTG



36051		CTGTGACTGG GACACTGACC		TCTGAGAATA AGACTCTTAT
36101		CGACCGAGTT GCTGGCTCAA		
36151		TAGCAGAACT ATCGTCTTGA		
36201		AACTCTCAAG TTGAGAGTTC		
36251		CGTGCACCCA GCACGTGGGT	•	
36301		GTGAGCAAAA CACTCGTTTT		 AAAAAAGGGA TTTTTTCCCT
36351		CACGGAAATG GTGCCTTTAC		
36401	· · · · · · · · · · · · · · · · · · ·	ATTTATCAGG TAAATAGTCC		
36451		GAAAAATAAA CTTTTTATTT		
36501		CTGACGTCTA GACTGCAGAT		
36551		CGTATCACGA GCATAGTGCT		

PacI

36601 ATTCTTAATT TCTTAATTAA (SEQ ID NO:34)
TAAGAATTAA AGAATTAATT (SEQ ID NO:35)

Ligure 27 AM

VIRUS (P5)	PLASMID	VIRUS (P	21)
MRKAc5gag(E3*) MRKAc5gag(E3*) 1 Kb*ladder	pAd5MRKgagSPA(E3*) pAd5MRKmCMVgag(E3*)	MRKAd5gag(E3·) MRKAd5gagSPA(E3•)	, MRKAd5mCMVgag(E3+)

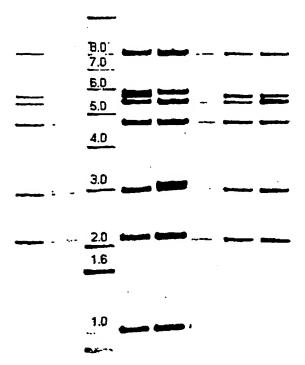


FIGURE 28

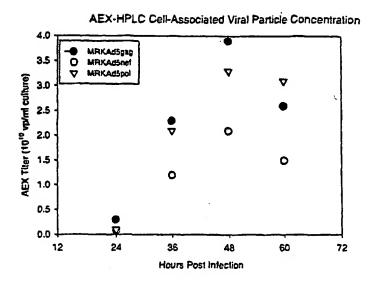


FIGURE 29A

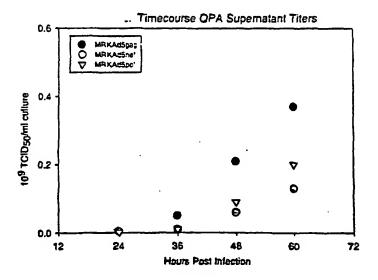


FIGURE 29B

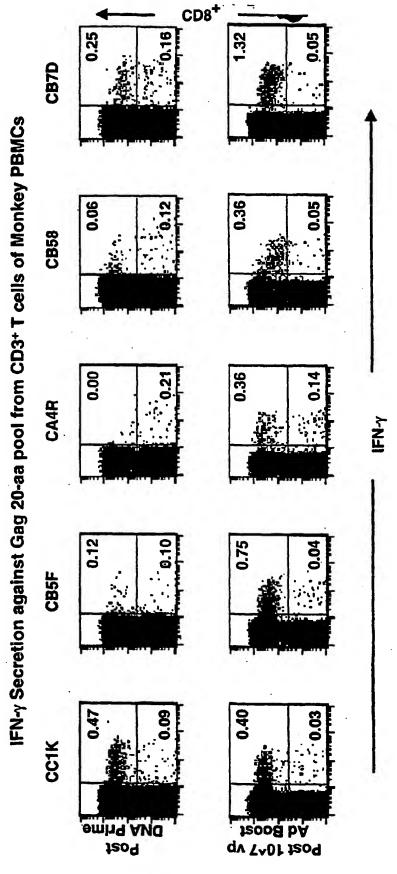
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gca Ala	gtc Val	ttc Phe	gtt Val 20	tcg Ser	ccc Pro	agc Ser	gag Glu	atc Ile 25	tcc Ser	att Ile	gtg Val	tgg Trp	gcc Ala 30	tcc Ser	agg Arg	96
gag Glu	ctg Leu	gag Glu 35	agg Arg	ttt Phe	gct Ala	gtg Val	aac Asn 40	cct Pro	ggc Gly	ctg Leu	ctg Leu	gag Glu 45	acc Thr	tct Ser	gag Glu	144
ggg Gly	tgc Cys 50	agg Arg	cag Gln	atc Ile	ctg Leu	ggc Gly 55	cag Gln	ctc Leu	cag Gln	ecc Pro	tec Ser 60	ctg Leu	caa Gln	aca Thr	Gly ggc	192
tct Ser 65	gag Glu	gag Glu	ctg Leu	agg Arg	tcc Ser 70	ctg Leu	tac Tyr	aac Asn	aca Thr	gtg Val 75	gct Ala	acc Thr	ctg Leu	tac Tyr	tgt Cys 80	240
gtg Val	cac His	cag Gln	aag Lys	att Ile 85	gat Asp	gtg Val	aag Lys	gac Asp	acc Thr 90	aag Lys	gag Glu	gcc Ala	ctg Leu	gag Glu 95	aag Lys	288
att Ile	gag Glu	gag Glu	gag Glu 100	cag Gln	aac Asn	aag Lys	tcc Ser	aag Lys 105	aag Lys	aag Lys	gcc Ala	cag Gln	cag Gln 110	gct Ala	gct Ala	336
gct Ala	ggc Gly	aca Thr 115	Gly	aac Asn	tcc Ser	agc Ser	cag Gln 120	gtg Val	tcc Ser	cag Gln	aac Asn	tac Tyr 125	ccc Pro	att Ile	gtg Val	384
cag Gln	aac Asn 130	ctc Leu	cag Gln	ggc	cag Gln	atg Met 135	gtg Val	cac His	cag Gln	gcc Ala	atc Ile 140	tcc Ser	Pro	cgg	acc Thr	432
ctg Leu 145	Asn	gcc Ala	tgg Trp	gtg Val	aag Lys 150	gtg Val	gtg Val	gag Glu	gag Glu	aag Lys 155	Ala	ttc Phe	tcc Ser	cct Pro	gag Glu 160	480
gtg Val	atc	ccc Pro	atg Met	ttc Phe 165	Ser	gcc	ctg Leu	tct Ser	gag Glu 170	Gly	gcc Ala	acc Thr	Pro	cag Gln 175	Asp	528
ctg Lev	aac Asn	acc	atg Met 180	Leu	aac Asn	aca Thr	gtg Val	ggg Gly 185	Gly	cat His	cag Gln	gct Ala	gcc Ala 190	Met	Gln	576
atg Met	ctg Leu	aag Lys 195	Gl v	acc	atc	aat Asn	gag Glu 200	Glu	gct Ala	gct	gag Glu	tgg Trp 205	Asp	agg Arg	ctg Leu	624
cat His	Pro 210	Val	cac His	gct	Gly	Pro 215	Ile	gcc Ala	ccc Pro	Gly	Gln 220	Met	agg Arg	gag Glu	Pro	672
agg Arg 225	Gly	tct Ser	gac Asp	att	gct Ala 230	G1y	acc Thr	acc Thr	tcc Ser	Thr 235	Lev	cag Gli	gag Glu	cac Glr	att ille 240	720
G17 ggd	tgg Trp	atg Met	acc	Asn 245	ASD	Pro	ecc Pro	ato	e cct Pro 250	Val	. Gly	g gaa gaa gaa	ato Ile	tac Tyr 255	aag Lys	768

Figure 30'A"

agg Arg	tgg Trp	atc Ile	atc Ile 260	ctg Leu	Gly	ctg Leu	aac Asn	aag Lys 265	att Ile	gtg Val	agg Arg	atg Met	tac Tyr 270	tcc Ser	ccc Pro	816
acc Thr	tcc Ser	atc Ile 275	ctg Leu	gac Asp	atc Ile	agg Arg	cag Gln 280	ggc Gly	ccc Pro	aag Lys	gag Glu	Pro 285	ttc Phe	agg Arg	gac Asp	864
tat Tyr	gtg Val 290	gac Asp	agg Arg	ttc Phe	tac Tyr	aag Lys 295	acc Thr	ctg Leu	agg Arg	gct Ala	gag Glu 300	cag Gln	gcc Ala	tcc Ser	cag Gln	912
gag Glu 305	gtg Val	aag Lys	aac Asn	tgg Trp	Met 310	aca Thr	Gjn gag	acc Thr	ctg Leu	ctg Leu 315	gtg Val	cag Gln	aat Asn	gcc Ala	aac Asn 320	960
cct Pro	gac Asp	tgc Cys	aag Lys	acc Thr 325	atc Ile	ctg Leu	aag Lys	gcc Ala	ctg Leu 330	Gly ggc	cct Pro	gct Ala	gcc Ala	acc Thr 335	ctg Leu	1008
gag Glu	gag Glu	atg Met	atg Met 340	aca Thr	gcc Ala	tgc Cys	cag Gln	999 Gly 345	gtg Val	GJA aaa	ggc	ect Pro	ggt Gly 350	cac His	aag Lys	1056
gcc Ala	agg Arg	gtg Val 355	ctg Leu	gct Ala	gag Glu	gcc Ala	atg Met 360	tcc Ser	cag Gln	gtg Val	acc Thr	aac Asn 365	tcc Ser	gcc Ala	acc Thr	1104
atc Ile	atg Met 370	atg Met	cag Gln	agg	ggc Gly	aac Asn 375	ttc Phe	agg A rg	aac Asn	cag Gln	agg Arg 380	aag Lys	aca Thr	gtg Val	aag Lys	1152
tgc Cys 385	ttc Phe	aac Asn	tgt Cys	ggc Gly	aag Lys 390	gtg Val	ggc Gly	cac His	att Ile	gcc Ala 395	aag Lys	aac Asn	tgt Cys	ágg Arg	gcc Ala 400	1200
Pro	agg Arg	aag Lys	aag Lys	ggc Gly 405	tgc Cys	tgg Trp	aag Lys	tgt Cys	ggc Gly 410	aag Lys	gag Glu	ggc Gly	cac His	cag Gln 415	atg Met	1248
aag Lys	gac Asp	tgc Cys	aat Asn 420	gag Glu	agg Arg	cag Gln	gcc Ala	aac Asn 425	ttc Phe	ctg Leu	GJy ggc	aaa Lys	atc Ile 430	tgg Trp	ccc Pro	1296
tcc Ser	cac His	aag Lys 435	Gly ggc	agg Arg	cct Pro	Gjy ggc	aac Asn 440	ttc Phe	ctc Leu	cag Gln	tcc Ser	agg Arg 445	cct Pro	gag Glu	ccc Pro	1344
aca Thr	gcc Ala 450	cct Pro	ccc Pro	gag Glu	gag Glu	tcc Ser 455	ttc Phe	agg Arg	ttť Phe	ejy 888	gag Glu 460	Glu	aag Lys	acc Thr	acc Thr	1392
ccc Pro 465	agc Ser	cag Gln	aag Lys	cag Gln	gag Glu 470	ccc Pro	att Ile	gac Asp	aag Lys	gag Glu 475	ctg Leu	tac Tyr	ccc Pro	ctg Leu	gcc Ala 480	1440
tcc Ser	ctg Leu	agg Arg	tcc Ser	ctg Leu 485	ttt Phe	ggc Gly	aac Asn	gac Asp	ccc Pro 490	tcc Ser	tcc Ser	cag Gln	taa •	(SII	NO:36)	1482

Figure 30 B

Figure 31



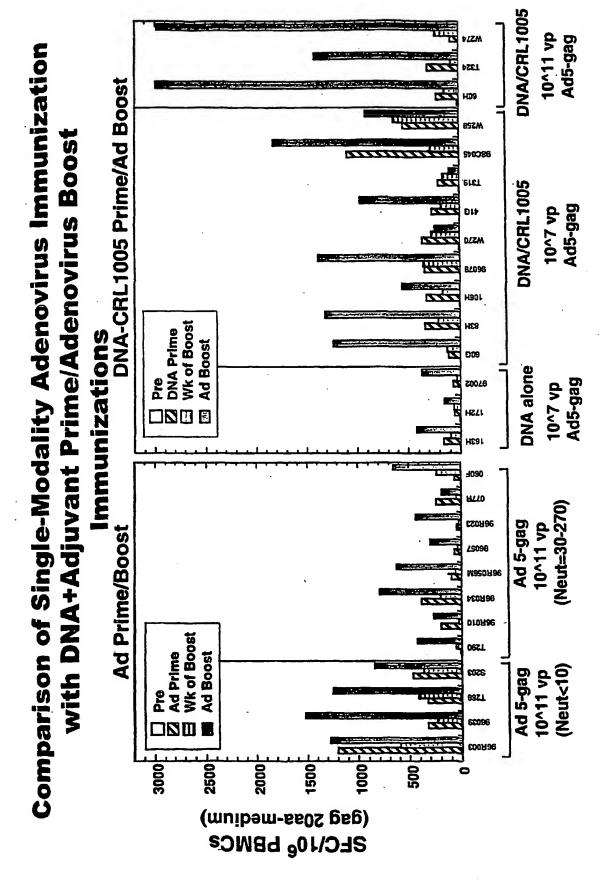


FIGURE 33A

ATGGGTGCTA	GGGCTTCTGT	GCTGTCTGGT	GGTGAGCTGG	ACAAGTGGGA	GAAGATCAGG
CTGAGGCCTG	GTGGCAAGAA	GAAGTACAAG	CTAAAGCACA	TTGTGTGGGC	CTCCAGGGAG
CTGGAGAGGT	TTGCTGTGAA	CCCTGGCCTG	CTGGAGACCT	CTGAGGGGTG	CAGGCAGATC
CTGGGCCAGC	TCCAGCCCTC	CCTGCAAACA	GGCTCTGAGG	AGCTGAGGTC	CCTGTACAAC
ACAGTGGCTA	CCCTGTACTG	TGTGCACCAG	AAGATTGATG	TGAAGGACAC	CAAGGAGGCC
CTGGAGAAGA	TTGAGGAGGA	GCAGAACAAG	TCCAAGAAGA	AGGCCCAGCA	GGCTGCTGCT
GGCACAGGCA	ACTCCAGCCA	GGTGTCCCAG	AACTACCCCA	TTGTGCAGAA	CCTCCAGGGC
CAGATGGTGC	ACCAGGCCAT	CTCCCCCGG	ACCCTGAATG	CCTGGGTGAA	GGTGGTGGAG
GAGAAGGCCT	TCTCCCCTGA	GGTGATCCCC	ATGTTCTCTG	CCCTGTCTGA	GGGTGCCACC
CCCCAGGACC	TGAACACCAT	GCTGAACACA	GTGGGGGGCC	ATCAGGCTGC	CATGCAGATG
CTGAAGGAGA	CCATCAATGA	GGAGGCTGCT	GAGTGGGACA	GGCTGCATCC	TGTGCACGCT
GGCCCCATTG	CCCCCGCCA	GATGAGGGAG	CCCAGGGGCT	CTGACATTGC	TGGCACCACC
TCCACCCTCC	AGGAGCAGAT	TGGCTGGATG	ACCAACAACC	CCCCCATCCC	TGTGGGGGAA
ATCTACAAGA	GGTGGATCAT	CCTGGGCCTG	AACAAGATTG	TGAGGATGTA	CTCCCCCACC
TCCATCCTGG	ACATCAGGCA	GGGCCCCAAG	GAGCCCTTCA	GGGACTATGT	GGACAGGTTC
TACAAGACCC	TGAGGGCTGA	GCAGGCCTCC	CAGGAGGTGA	AGAACTGGAT	GACAGAGACC
CTGCTGGTGC	AGAATGCCAA	CCCTGACTGC	AAGACCATCC	TGAAGGCCCT	GGGCCCTGCT
GCCACCCTGG	AGGAGATGAT	GACAGCCTGC	CAGGGGGTGG	GGGGCCCTGG	TCACAAGGCC
AGGGTGCTGG	CTGAGGCCAT	GTCCCAGGTG	ACCAACTCCG	CCACCATCAT	GATGCAGAGG
GGCAACTTCA	GGAACCAGAG	GAAGACAGTG	AAGTGCTTCA	ACTGTGGCAA	GGTGGGCCAC
ATTGCCAAGA	ACTGTAGGGC	CCCCAGGAAG	AAGGGCTGCT	GGAAGTGTGG	CAAGGAGGGC
CACCAGATGA	AGGACTGCAA	TGAGAGGCAG	GCCAACTTCC	TGGGCAAAAT	CTGGCCCTCC
CACAAGGGCA	GGCCTGGCAA	CTTCCTCCAG	TCCAGGCCTG	AGCCCACAGC	CCCTCCCGAG
GAGTCCTTCA	GGTTTGGGGA	GGAGAAGACC	ACCCCCAGCC	AGAAGCAGGA	GCCCATTGAC
	ACCCCCTGGC				
	TCTCCCCCAT				
	AGCAGTGGCC				
	AGAAGGAGGG				
-	CCATCAAGAA				
_	AGAGGACCCA				
					CTTCTCTGTG
					CAACAATGAG
					CTCCCCTGCC
					CCCTGACATT
					TEGECAGCAC
					CACCCTGAC
					CCCCGACAAG
					TGACATCCAG
					GGTGAGGCAG
					GACTGAGGAG
GCTGAGCTGG	AGCTGGCTGA	GAACAGGGAG	ATCCTGAAGG	AGCCTGTGCA	TGGGGTGTAC

FIGURE 33B

שאתכארנינים	CCAAGGACCT	СУЛЛССТСУС	ATCCAGAAGC	AGGGCCAGGG	CCAGTGGACC
	ACCAGGAGCC				
	CCAATGATGT				
TCCATTGTGA	TCTGGGGCAA	GACCCCCAAG	TTCAAGCTGC	CCATCCAGAA	GGAGACCTGG
GAGACCTGGT	GGACTGAGTA	CTGGCAGGCC	ACCTGGATCC	CTGAGTGGGA	GTTTGTGAAC
ACCCCCCCC	TGGTGAAGCT	GTGGTACCAG.	CTGGAGAAGG	AGCCCATTGT	GGGGGCTGAG
ACCTTCTATG	TGGCTGGGGC	TGCCAACAGG	GAGACCAAGC	TGGGCAAGGC	TGGCTATGTG
ACCAACAGGG	GCAGGCAGAA	GGTGGTGACC	CTGACTGACA	CCACCAACCA	GAAGACTGCC
CTCCAGGCCA	TCTACCTGGC	CCTCCAGGAC	TCTGGCCTGG	AGGTGAACAT	TGTGACTGCC
TCCCAGTATG	CCCTGGGCAT	CATCCAGGCC	CAGCCTGATC	AGTCTGAGTC	TGAGCTGGTG
AACCAGATCA	TTGAGCAGCT	GATCAAGAAG	GAGAAGGTGT	ACCTGGCCTG	GGTGCCTGCC
CACAAGGGCA	TTGGGGGCAA	TGAGCAGGTG	GACAAGCTGG	TGTCTGCTGG	CATCAGGAAG
GTGCTGTTCC	TGGATGGCAT	TGACAAGGCC	CAGGATGAGC	ATGAGAAGTA	CCACTCCAAC
TGGAGGGCTA	TGGCCTCTGA	CTTCAACCTG	CCCCTGTGG	TGGCTAAGGA	GATTGTGGCC
TCCTGTGACA	AGTGCCAGCT	GAAGGGGGAG	GCCATGCATG	GGCAGGTGGA	CTGCTCCCCT
GGCATCTGGC	AGCTGGCCTG	CACCCACCTG	GAGGGCAAGG	TGATCCTGGT	GGCTGTGCAT
GTGGCCTCCG	GCTACATTGA	GGCTGAGGTG	ATCCCTGCTG	AGACAGGCCA	GGAGACTGCC
TACTTCCTGC	TGAAGCTGGC	TGGCAGGTGG	CCTGTGAAGA	CCATCCACAC	TGCCAATGGC
TCCAACTTCA	CTGGGGCCAC	AGTGAGGGCT	GCCTGCTGGT	GGGCTGGCAT	CAAGCAGGAG
TTTGGCATCC	CCTACAACCC	CCAGTCCCAG	GGGGTGGTGG	CCTCCATGAA	CAAGGAGCTG
AAGAAGATCA	TTGGGCAGGT	GAGGGACCAG	GCTGAGCACC	TGAAGACAGC	TGTGCAGATG
GCTGTGTTCA	TCCACAACTT	CAAGAGGAAG	GGGGGCATCG	GGGGCTACTC	CGCTGGGGAG
AGGATTGTGG	ACATCATTGC	CACAGACATC	CAGACCAAGG	AGCTCCAGAA	GCAGATCACC
AAGATCCAGA	ACTTCAGGGT	GTACTACAGG	GACTCCAGGA	ACCCCCTGTG	GAAGGGCCCT
GCCAAGCTGC	TGTGGAAGGG	GGAGGGGGCT	GTGGTGATCC	AGGACAACTC	TGACATCAAG
GTGGTGCCCA	GGAGGAAGGC	CAAGATCATC	AGGGACTATG	GCAAGCAGAT	GGCTGGGGAT
GACTGTGTGG	CCTCCAGGCA	GGATGAGGAC	TAA		
SEQ ID NO:	38				

FIGURE 34A

Met Gly Ala Arg Ala Ser Val Leu Ser Gly Gly Glu Leu Asp Lys Trp Glu Lys Ile Arg Leu Arg Pro Gly Gly Lys Lys Lys Tyr Lys Leu Lys His Ile Val Trp Ala Ser Arg Glu Leu Glu Arg Phe Ala Val Asn Pro Gly Leu Leu Glu Thr Ser Glu Gly Cys Arg Gln Ile Leu Gly Gln Leu Gln Pro Ser Leu Gln Thr Gly Ser Glu Glu Leu Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys Val His Gln Lys Ile Asp Val Lys Asp Thr Lys Glu Ala Leu Glu Lys Ile Glu Glu Glu Gln Asn Lys Ser Lys Lys Lys Ala Gln Gln Ala Ala Gly Thr Gly Asn Ser Ser Gln Val Ser Gln Asn Tyr Pro Ile Val Gln Asn Leu Gln Gly Gln Met Val His Gln Ala Ile Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val Val Glu Glu Lys Ala Phe Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln Ala Ala Met Gln Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg Leu His Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln Met Arg Glu Pro Arg Gly Ser Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile Gly Trp Met Thr Asn Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu Asn Lys Ile Val Arg Met Tyr Ser Pro Thr Ser Ile Leu Asp Ile Arg Gln Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala Glu Gln Ala Ser Gln Glu Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val Gln Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala Ala Thr Leu Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly Pro Gly His Lys Ala Arg Val Leu Ala Glu Ala Met Ser Gln Val Thr Asn Ser Ala Thr Ile Met Met Gln Arg Gly Asn Phe Arg Asn Gln Arg Lys Thr Val Lys Cys Phe Asn Cys Gly Lys Val Gly His Ile Ala Lys Asn Cys Arg Ala Pro Arg Lys Lys Gly Cys Trp Lys Cys Gly Lys Glu Gly His Gln Met Lys Asp Cys Asn Glu Arg Gln Ala Asn Phe Leu Gly Lys Ile Trp Pro Ser His Lys Gly Arg Pro Gly Asn Phe Leu Gln Ser Arg Pro Glu Pro Thr Ala Pro Pro Glu Glu Ser Phe Arg Phe Gly Glu Glu Lys Thr Thr Pro Ser Gln Lys Gln Glu Pro Ile Asp Lys Glu Leu Tyr Pro Leu Ala Ser Leu Arg Ser Leu Phe Gly Asn Asp Pro Ser Ser Gln Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Ser Val Thr Val Leu Ala Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro

FIGURE 34B

Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Cly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly Jle Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp SEQ ID NO: 39

International application No.

			PC17US01/28861	· l			
	SSIFICATION OF SUBJECT MATTER	 					
IPC(7)	: C12N 15/86 : 435/456						
US CL	According to International Patent Classification (IPC) or to both national classification and IPC						
Minimum do	cumentation searched (classification system followed	by electification mod	hale)				
	24/205.1, 207.1, 227.1, 233.1; 435/69.1, 69.3, 173.						
0.0 42	24200. 1, 201. 1, 221. 1, 233. 1, 433.05. 1, 05.3, 113.	5, 255.1, 526.1, 400,	550,25.72,				
Documentation	on searched other than minimum documentation to the	e extent that such doca	ments are include	d in the fields searched			
				-			
							
Electronic da	ta base consulted during the international search (nan	ne of data base and, w	here practicable.	earch terms used)			
	ontimuation Sheet		and production,				
C. DOC	UMENTS CONSIDERED TO BE RELEVANT						
Category *	Citation of document, with indication, where ap	monriate of the rele	vent necesors	Relevant to claim No.			
X	WO 96/39178 (ERTL et al.) 12 December 1996 (12			1-3, 8-11, 18			
	and claims 1 and 5.		, 0,10, 12, 15	1-5, 0-11, 10			
Y				4, 5, 13-17, 29-32, 34, 35, 37			
x	US 6,019,978 A (ERTL et al.) 1 February 2000,(01/02/2000), see columns 2, 7 and 8.						
Y	4, 5, 13-17, 29-32, 34, 35, 37						
X,P	US 6,287,571 B (ERTL et al.) 11 September 2001 (11/09/2001), see columns 2, 7, 8 2, 1, 9, 18 2, 18						
x	US 5,643,579A (HUNG et al.) 1 July 1997 (01/07/	1997), see examples 1	, 2, 25 and 26.	1-3, 8, 9-11, 18			
Ÿ				4,5,13-17, 29-32, 34, 35, 37			
Y	WANG et al. The use of an E1-deleted, replication	-defective adenovirus	recombinant	1-3, 9-11, 13-18			
•	expressing the rabies virus glycoprotein for early vi			1,0,7,11,12,12			
	Journal of Virology (March 1997) Vol. 71, No. 5,						
M sunt	do-mark and listed in the continuous of Par C	<u> </u>	6il	<u> </u>			
	r documents are listed in the continuation of Box C.	-	family annex.				
• s	pecial categories of cited documents:			nternational filing date or h the application but cited to			
	t defining the general state of the art which is not considered to			inderlying the invention			
oe or par	ticular relevance			ne claimed invention earnot be			
"E" earlier ap	pplication or patent published on or after the international filing		novel or cannot be considered	dered to involve an inventive			
	t which may throw doubts on priority claim(s) or which is cited ish the publication date of another citation or other special reason			he claimed invention cannot be step when the document is			
(23 specia		combined w	ith one or more other s	sch documents, such			
O documen	t referring to an oral disclosure, use, exhibition or other means		being obvious to a per				
	u published prior to the international filing date but later than the	*&* document m	ember of the same pate	nt family			
priority.	date claimed	.					
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Box	PCT	Ulrike Winkler, Ph	i.D. // Com	, _ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \			
	ahington, D.C. 20231 o. (703)305-3230	Telephone No. 703	-308-0196	1)			
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Form PCT/ISA/210 (second sheet) (July 1998)

International application No.

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ategory *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.			
Y .	NATUK et al. Immunogenicity of recombinant human adenovirus -human immunodeficiency virus vaccines in chimpanzees. Aids Research and Human Retroviruses (1993) Vol. 9, No. 5, pp395-404, see material and methods.	1, 9, 29-32			
Y	PREVEC et al. Immune response to HIV-1 gag antigens induced by recombinant adenovirus vectors in mice and rhesus macaque monkeys. Journal of Acquired Immune Deficincy Syndrome. (1991) Vol. 4, No. 6 pp. 568-76, see abstract.	1, 9, 29-32			
Y	LORI et al. Rapid protection against human immunodeficiency virus type 1 (HIV-1) replication mediated by high efficiency non-retroviral delivery of genes interfering with HIV-1 tat and gag. Gene Therapy (1994) Vol. 1, No. 1, pp. 27-31, see abstract.				
Y	PFARR et al. Differential effects of polyadenylation regions on gene expression in mammalian cells. DNA (1986) Vol. 5, No. 2, pp.115-22, see abstract.	16			
Y	NATUK et al. Adenovirus vectored vaccine. Developmental Biological Standards (1994) Vol. 82, pp. 71-77, see abstract.	1, 9			
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International application No.

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Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)
This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:
Claim Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
2. Claim Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
. 3. Claim Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).
Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)
This International Searching Authority found multiple inventions in this international application, as follows: Please See Continuation Sheet
As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
 No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-5, 8-11, 13-18, 29-32, 34, 35, 37 Remark on Protest
No protest accompanied the payment of additional search fees.

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This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group	Claims	<u> </u>
1	1-5, 8-11, 13-18, 29, 30, 31, 32, 34, 35, 37	The claims are directed to an adenoviral vector that is at least partially deleted of <u>AE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Gag protein (SEQ ID NO: 29) inserted in the parallel orientation of E1. In addition the vector contains a promoter and a polyadenylation signal.
2	6, 7, 36	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Gag protein (SEQ ID NO: 29).
3	12, 33	The claims are directed to an adenoviral vector that is at least partially deleted of ΔEI , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV protein inserted in the antiparallel orientation of E1.
4	19-23, 38-42	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Gag protein.
5	24, 27, 28, 43, 46, 47	The claim is directed to a method of generating a cellular mediated immune response to HIV Gag protein with the recombinant adenoviral particle.
6	25, 26, 44, 45	The claim is directed to a method of generating a cellular mediated immune response to HIV Gag protein with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.
7	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of <u>AE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 1)</u> inserted in the parallel orientation of E1.
8	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of ΔE_1 , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in the parallel orientation of E1.
9	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of <u>AE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 7)</u> inserted in the parallel orientation of E1.
10	52	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in the antiparallel orientation of E1.
11	52	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in the antiparallel orientation of E1.
12	52	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE_1 , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in the antiparallel orientation of E1.
		The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$

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		and ΔΕ3, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1)
	·	inserted in E1.
14	55	The claim is directed to an adenoviral vector that is at least partially deleted of ΔΕ1 and ΔΕ3, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5)
		inserted in E1.
15	.55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$
1.5	1.55	and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7)
		inserted in E1.
16	57-61	The claims are directed to a method of making and harvesting of a recombinant
		adenoviral particle that contains a gene encoding an HIV Pol protein. The claim is directed to a method of generating a cellular mediated immune response
17	62, 65, 66	to HIV Pol protein with the recombinant adenoviral particle.
18	63, 64	The claim is directed to a method of generating a cellular mediated immune response
10	05, 04	to HIV Pol protein with the recombinant adenoviral particle in addition to
	1	administering a DNA plasmid vaccine.
19	67-70, 72,	The claims are directed to an adenoviral vector that is at least partially deleted of
	73, 75	ΔΕ1, the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9)
		inserted in the parallel orientation of E1.
20	67-70, 72,	The claims are directed to an adenoviral vector that is at least partially deleted of
	73, 75	ΔΕ1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11)
		inserted in the parallel orientation of E1.
21	67-70, 72,	The claims are directed to an adenoviral vector that is at least partially deleted of
21	73, 75	ΔΕ1, the vector contains the cis-acting packaging sequence of the wild type
	1 /3, /3	adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13)
		inserted in the parallel orientation of E1.
22	67-70, 72,	The claims are directed to an adenoviral vector that is at least partially deleted of
	73, 75	Δ E1, the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15)
		inserted in the parallel orientation of E1.
23	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus
	1	genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in
		the antiparallel orientation of E1.
24	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$,
	1	the vector contains the cis-acting packaging sequence of the wild type adenovirus
	1.	genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in
		the antiparallel orientation of E1.
25	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$,
	1	the vector contains the cis-acting packaging sequence of the wild type adenovirus
		genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in
26		the antiparallel orientation of E1. The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$.
20	71	the vector contains the cis-acting packaging sequence of the wild type adenovirus
	İ	genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in
		the antiparallel orientation of E1.
27	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$
		and AE3, the vector contains the cis-acting packaging sequence of the wild type
	-	adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9)
		inserted in E1.
28	74	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE_1
	}	and ΔΕ3, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11)
		inserted in E1.
29	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$

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		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in E1.
30	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in E1.
31	76-80	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Nef protein.
32	81, 84, 85	The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle.
33	82, 83	The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.
34	86a	The claim is drawn to a multivalem vaccine wherein gag, pol and nef are expressed from three individual vectors.
35	86b, 88, 89	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from one individual vectors.
36	86c, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from two individual vectors, one expressing nef-pol fusion and one expressing gag.
37	86d, 87, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from two individual vectors, one expressing gag-pol fusion and one expressing nef.
38	86e, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from two individual vectors, one expressing nef-gag fusion and one expressing pol.
39	86f, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from a single vectors as a fusion protein.
40	86g, 88	The claims are drawn to a multivalent vaccine wherein gag and pol are expressed from two individual vectors.
41	86h, 88, 89	The claims are drawn to a multivalent vaccine wherein gag and pol are expressed individually from one vector.
42	86i, 88	The claims are drawn to a multivalent vaccine wherein pol and nef are expressed from two individual vectors.
43	86j, 88, 89	The claims are drawn to a multivalent vaccine wherein pol and nef are expressed from individually from one vector.
44	86k, 88	The claims are drawn to a multivalent vaccine wherein nef and gag are expressed individually from one vector.
45	861, 88, 89	The claims are drawn to a multivalent vaccine wherein nef and gag are expressed individually from one vector.
46	86m, 88	The claims are drawn to a multivalent vaccine wherein gag and pol are expressed as a fusion protein from one vector.
47	86n, 88	The claims are drawn to a multivalent vaccine wherein pol and nef are expressed as a fusion protein from one vector.
48	860, 88	The claims are drawn to a multivalent vaccine wherein nef and gag are expressed as a

The inventions listed as Groups 1-48 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The technical feature linking groups 1-33 appears to be a recombinant adenoviral vector wherein the adenoviral vector is at least partially deleted in E1 but the vector may contain more deletions, the vector contains wild type sequences including packaging signals and a gene encoding a heterologous HIV protein or fragments thereof. Erd et al. (WO 96/39178) disclose a recombinant adenoviral vector that is deleted in E1 and partially deleted in E3, the remainder of the adenoviral vector contains wild type sequences. The vector additionally contains an insertion of a heterologous protein which includes HIV proteins (see abstract and claims 1 and 5). Therefore, the technical feature linking the inventions of groups 1-45 does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art.

The special technical feature of the following groups 1-3, 7-15, 19-30 and 34-48 is considered to be the combination of sequences that is disclosed in each group, see individual claim groupings above for the different sequences. The DNA disclosed in each group is made up of a different sequence having a different structure and different function.

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The special technical feature of group 4, 16 and 31 is considered to be a method of producing recombinant adenoviral particles. Each group contains different sequences hence the resulting particles would have different structures and functions associated with the particle.

The special technical feature of group 5, 17 and 32 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors. Each group contains different sequences a encoding different protein, therefore the resulting immune response will also be different.

The special technical feature of group 6, 18 and 33 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors in conjunction with immunizing the individual a DNA plasmid vaccine. Each method contains different sequences encoding a different protein, therefore the resulting immune response will also be different.

Accordingly, groups 1-48 are not so linked by the same or corresponding technical feature as to form a single general inventive concept.

Continuation of B. FIELDS SEARCHED Item 3:

WEST 2.0, STN-BIOSIS, MEDLINE

adenoviral vector, deletion, HIV, Gag, polyadenylation signal, CMV promoter